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Fluctuations of Chlorophyll and Related Physical Parameters in British Columbia Coastal Waters

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WIEGAND, R. C., AND S. POND. 1979. Fluctuations of chlorophyll and related physical parameters in British Columbia coastal waters. J. Fish. Res. Board Can. 36: 113–121.

Continuous horizontal profiles of chlorophyll, temperature, salinity, and oxygen were made at three depths and in three locations in British Columbia coastal waters, and spectra and cross spectra calculated. Generally, fairly strong relationships exist between chlorophyll and temperature and salinity, especially at scales of order 10–1000 m. However, the relationships are not always expressed through the same physical parameter. It is apparent from coherence and phase data that in a turbulent environment the vertical profiles of the parameters are important in determining the nature of the relationships. Average spectra show power law relationships similar to the results of other authors.

Key words: chlorophyll, temperature, salinity, oxygen fluctuations; surface coastal waters; T, S, O_2, chlorophyll fluctuations; spectra and cross-spectra of T, S, O_2, chlorophyll; measurements of T, S, O_2, chlorophyll in surface waters

WIEGAND, R. C., AND S. POND. 1979. Fluctuations of chlorophyll and related physical parameters in British Columbia coastal waters. J. Fish. Res. Board Can. 36: 113–121.

Nous avons enregistré des profils horizontaux continus de la chlorophylle, de la température, de la salinité et de l'oxygène à trois profondeurs et à trois endroits des eaux côtières de la Colombie-Britannique, et calculé des spectres et des spectres croisés. Il existe en général des relations assez étroites entre la chlorophylle et la température et la salinité, surtout aux échelles de l'ordre de 10–1000 m. Cependant, ces relations ne s'expriment pas toujours par le même paramètre physique. Il est évident, d'après les données de cohérence et de phase, que dans un milieu turbulent, les profils verticaux des paramètres sont importants quand il s'agit de déterminer la nature des relations. Les spectres moyens exhibent des relations « power law » semblables aux résultats d'autres auteurs.

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WITHIN the last few years there has been a renewed interest in the distribution of phytoplankton in the oceans. One reason is the importance of plant life in the marine food chain; another is that heterogeneity is thought to play some role in the stability of the system (Steele 1974).

Seafarers and naturalists made early observations of patchiness (Bainbridge 1957) but most people believed in a uniform plankton distribution. Although Herdman (1907) became aware of both spatial and temporal variations in planktonic distributions, controversy continued (Hardy 1935). Further proof of heterogeneity came with the use of the Hardy plankton recorder in tows across the North Sea (Lucas 1941).

More recent studies of phytoplankton abundance are concerned with the total distribution as indicated by chlorophyll fluorescence. These studies show relationships with hydrographic parameters (Lorenzen 1971; Platt et al. 1970). Most intriguing, however, is the hypothesis of a coupling between the phytoplankton distribution and that of turbulent energy (Platt 1972; Denman and Platt 1975). These last two references present data in which the spectrum of chlorophyll *a* fluctuations is approximately proportional to $k^{-5/3}$, characteristic of homogeneous isotropic turbulence (k is the radian wavenumber $= 2\Pi/\text{wavelength}, \lambda$). Using temperature as an "indicator" of the turbulent processes, Denman and Platt (1975) infer from high values in the chlorophyll–temperature coherency spectrum that at some scales the distribution of chlorophyll is largely determined by physical rather than biological processes. Other authors concur with these observations (Fasham and Pugh 1976; Powell et al. 1975).

Wiegand (1976) examined the spatial heterogeneity of phytoplankton as indicated by chlorophyll *a* in the surface region of an estuarine environment. In addition, temperature, salinity, oxygen, and velocity were measured. Data were collected in Indian Arm, Howe Sound, and the Strait of Georgia in the vicinity of the Fraser River plume, at three depths of approximately 1, 3, and 5 m. The locations (Fig. 1) were visited during the months of April through July 1973, roughly coinciding with the spring phytoplankton bloom and its decline. Observations were made at approximately 1-mo intervals. In this paper, some results from Indian Arm will be described and then averages of all the results will be presented.

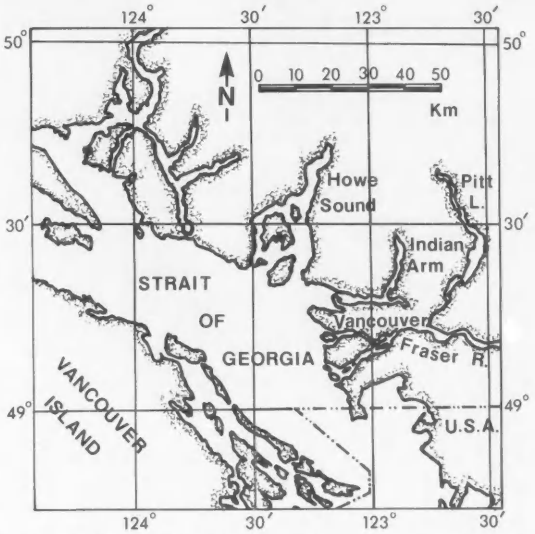

FIG. 1. Geographical locations of the study areas, taken from Canadian Hydrographic chart L3001.

Materials and Methods

Water at a given depth was pumped on board ship where temperature, oxygen, salinity, and chlorophyll were measured. In addition, temperature and the relative ship-water speed were measured in situ.

Sampling was done using a 373-W (0.5-HP), six-stage submersible deep-well pump. The pump, hose, and cables to the in situ instruments were housed in a 10-cm diam aluminum pipe with portals to access the pump intake and to allow the cables to be attached to the instruments. This pipe was attached to a crane which enabled placement outside the ship's bow wave. During the experiments the ship's speed was about $1 \pm 0.2\ \text{m}\cdot\text{s}^{-1}$ (2 knots). Since the pumped water had a speed in the hose of about $2\ \text{m}\cdot\text{s}^{-1}$ all the structure present could be sampled. Black rubber sandblast hose approximately 30 m long with 1-cm walls and a 2-cm orifice was used to transport the water on board.

The onboard instrument holder containing temperature, oxygen, and salinity probes was made of transparent acrylic plastic so that the sensors could be monitored for fouling. A 1-m section of hose was placed between the holder and the fluorometer so that light entering the system at the holder would not affect the measurement of chlorophyll.

Salinity was measured with a Yellow Springs Instrument Co. (YSI) model 33 S-C-T meter and probe modified to allow continuous measurement. Temperature corrections were applied to obtain salinity from conductivity, with a worse case error of $<5\%$. Details of the modifications and corrections are in Wiegand (1976). As the frequency response of the sensing unit appears to be better than the 1 Hz cutoff frequency used for filtering the data, the salinity spectral results should accurately describe in situ fluctuations other than the smoothing effects at high frequencies caused by the pumping system.

Thermistors in Wheatstone bridges were used to measure temperature. The output voltage of a bridge was linearly proportional to temperature over the range measured. By measuring temperature at the pump intake and in the instrument holder, the travel time of the water in the pumping system could be determined. Also, a comparison of spectral results allows an estimation of mixing or smoothing in the pumping system. From results presented later, it appears that the effective time constant of the thermistors is about 0.3 s.

Oxygen was measured with a YSI model 54 oxygen meter and model 5419 oxygen–temperature probe. For continuous recording the amplified meter voltage was used. Compensation for temperature effects on solubility of oxygen was

done electronically by the instrument; compensation for salinity effects and membrane aging was done during data processing as described in Wiegand (1976). A comparison of mean values of oxygen determined using the probe and results of Winkler titrations of discrete samples taken at approximately the same time and depth showed that the probe measured oxygen quite well (Wiegand 1976). Results of tests of other similar probes (National Oceanographic Instrumentation Center 1972) suggest a time constant of 5–8 s.

Fluorometry (Yentsch and Menzel 1963) was used to measure chlorophyll continuously. A Turner model III fluorometer equipped with a red-sensitive photomultiplier and a high volume continuous door was used. The filters were a Corning CS5-60 for the excitation lamp and a CS2-64 for the photomultiplier (Strickland and Parsons 1972). The general purpose ultraviolet lamp was used with allowances made for its exceptionally long warm-up period (Krauel 1972). From the manufacturer's figures, a time constant for the fluorometer is about 2–3 s.

Calibration of the instrument was done in the laboratory using cultured cells and comparing the output of the fluorometer to spectrophotometrically determined pigment content (Strickland and Parsons 1972). Chlorophyll–voltage relationship varied with species in agreement with Loftus et al. (1972). Because *Skeletonema costatum* is the dominant species during the spring and early summer (Parsons et al. 1970) the coefficients from its calibration were used.

The mean ship-water speed was measured with a General Oceanics model 2031 digital flowmeter and 2035 flowmeter readout. A calibration check was done in a hydraulics laboratory with the error in speed $<3\%$.

Analysis Technique

Statistical quantities such as variance, covariance, and spectra were computed using appropriate sums and products of the Fourier coefficients calculated from the data based on procedures given in Jenkins and Watts (1968), Bendat and Piersol (1971), and Platt and Denman (1975).

Signal Processing

The signals from the instruments were amplified and known DC voltages removed (to ensure maximum signal to noise ratio of the fluctuations) before recording on magnetic tape. Selected records were low pass filtered with a ½-power frequency of about 1 Hz to prevent aliasing before digitizing. The analogue signal was digitized with a sampling frequency of 4 Hz, and divided into sections of 1024 points (called blocks) to be Fourier transformed. Spectral analysis was done on several sequential blocks of data. The range of periods is about 256–0.5 s.

It is expected that turbulent fluctuations in the speed were much less than the mean of about 1 ms^{-1} so that Taylor's hypothesis, that is, length (λ) = mean speed of flow ($\bar{c}$) × period (Hinze 1959), can be used to convert the periods to length scales of about 256–0.5 m. However, because of the response limits of some of the instruments, the effects of the pump, and the approach to the system noise level at high frequencies, the smallest scale that can be discussed with confidence is 5–10 m.

Larger scales were examined by Fourier transforming the block averages (Garrett 1970) for data with 10 or more blocks. When plotted, the spectral estimates from this analysis are noticeable as they lack confidence bands. Although error bars could be constructed by assuming a distribution for the estimates, this procedure is inconsistent with the smaller scale results. These estimates cannot be used to examine details but do indicate trends in the large-scale behavior of the parameters. The coherence and phase for these estimates were not plotted for individual runs since they are not statistically significant. For convenience, spectral quantities calculated using block averages will be described as LS (large-scale) statistics.

The 68% confidence bands for the spectra are based on the observed variability in the estimates between blocks and the assumption that the squares of the Fourier amplitudes have a chi-squared distribution. Bars shown on the coherence plots for individual runs represent a region in which there is a 95% chance that the true coherence lies. They were calculated from the formula in Bendat and Piersol (1971). The 95% confidence bars for the phase are based on the formula given in Jenkins and Watts (1968).

The method used to determine significance levels for coherence is explained in Groves and Hannan (1968). Based on their formula, a 95% significance line is shown on each coherence plot. The value of coherence between two entirely random signals will be below this line 95% of the time.

Because of the wide range of frequencies used in the analysis it is convenient to use a log scale on the frequency axis. To show the relative contributions to the variance from different frequencies, $f\Phi$ is plotted against log f, where $\Phi(f)$ is the spectrum. If the spectral levels vary over a number of decades then log $f\Phi$ is plotted. Such a plot also enables us to look for power law relationships. The area under the curve is no longer proportional to the variance but it is still clear where the main contributions to the variance occur. In comparing our results with those obtained by other authors, note that Φ or log Φ instead of $f\Phi$ or log $f\Phi$ are often plotted.

Care must be exercised when producing representative average spectra, coherence, and phase spectra because of the phase relationships of individual runs. The rationale and a description of the methods used in averaging are given in Wiegand (1976) and a summary follows.

Normalization of the Fourier coefficients using the total variance was done, followed by multiplication of co- or quad-spectral quantities by -1 where necessary to ensure the phase of all spectra was around 0. Coherence and phase were calculated for each run and linearly averaged over the total number of runs to obtain averages for them. For the LS statistics, average co- and quad-spectra were first obtained and then coherence and phase calculated because of the small number of degrees of freedom in the LS spectral quantities for an individual run.

Results

Limitations Due to Pumping and the Signal to Noise Ratio

Some effects of pumping can be found by plotting the ratio of the onboard and in situ spectra of temperature, $\Phi_{TEMP}/\Phi_{T\text{-}OUT}$. Fluctuations produced by mixing in the hose should be at scales of order 10 times its diameter and the frequencies are smaller by about a factor of 5 than the ½ power or 3 dB point of the analog filters

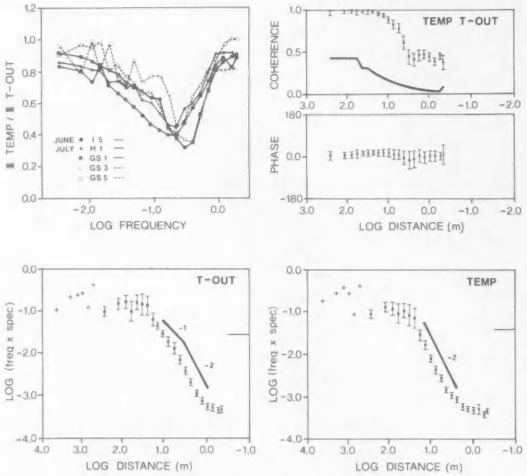

FIG. 2. Plot of the gain factor for the pump–hose system for some of the data runs. (*top*) Temperature coherence and phase spectra for June Indian Arm 5-m (Ind 5) data. Solid line is the 95% significance level and vertical bars represent 95% confidence intervals; Spectrum of the in situ temperature sensor (T-OUT) for the June Ind 5 data including LS results. (*bottom*) Spectrum of the on-board temperature sensor for the June Ind 5 data including LS results. For the spectra vertical bars represent 68% confidence limits for the mean. Each spectrum has been normalized; to show absolute levels of spectra reference lines corresponding to an $f\Phi$ value of 10^{-3} have been placed on each plot.

used to prepare the signals for digitization. Therefore, the gain factor ($\Phi_{\text{TEMP}}/\Phi_{\text{T-OUT}}$) should only show the smoothing due to mixing within the hose. Figure 2 contains plots of gain factors for some of the data runs. At very large scales the expected value of gain is 1. At the low frequencies, the power gain is within 20% of this value. Averaged over the runs it is about 0.9, suggesting that there is some loss of signal (10% in spectral level or 5% in amplitude) perhaps due to heat exchange with the walls of the hose and/or errors in calibration of the thermistors. For salt, chlorophyll, and oxygen, exchange with the walls should be negligible. The shapes of the curves in Fig. 2 are generally similar to that of a low pass filter with a 3 dB point (spectral ratio = ½) at roughly 10 m ($\log f = -1.0$ or $f = 0.1$ Hz and therefore $\lambda \simeq 10$ m). The gain passes through a minimum and rises to a value of ~ 1 at high frequencies suggesting equal noise at high frequencies.

The temperature spectra for the June Indian Arm 5-m data are also shown in Fig. 2. The larger scales, down to about 20–30 m, are similar; then the onboard spectrum (TEMP) falls more rapidly probably because of the filter effect of the pump and hose system. At about 5 m the slope of the outboard temperature spectrum (T-OUT) becomes steeper, probably due to the response limits of the thermistor. At the very small

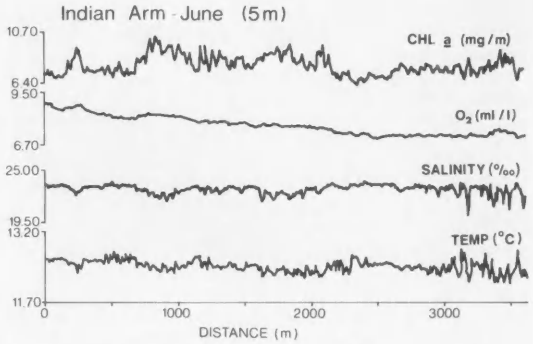

FIG. 3. June Indian Arm 5 data plotted against distance (distance $= \bar{c}t$ where $\bar{c} = .88$ m·s^{-1} for these data).

scales, both spectra become nearly flat and the spectral ratio approaches 1 since both spectra have nearly the same level due to the signal to noise limits of the data logging system.

The shape of the coherence spectrum for the same data (Fig. 2) is similar to that of the gain factor and is generally what one would expect, except perhaps for the relatively high coherences at small scales. The noise introduced during recording is in part the result of variations in the speed of the tape in a band near 1 Hz and this tape speed variation noise is coherent. Thus at high frequencies (small scales) two signals, including the noise, might appear to be coherent, partially coherent, or incoherent.

Pumping speed variations were as much as 20% of the mean but because of the averaging used in arriving at the spectral results, because the spectra were plotted against log distance, and because the sensors were at essentially the same point in the hose, this speed variation is not likely to affect the results noticeably.

Examination of the data collection system reveals that one can attach little meaning to the results at scales smaller than about 5–10 m and values for scales <5 m are not shown in subsequent figures.

INDIAN ARM DATA FROM 5-M DEPTH IN JUNE

The time series data from Indian Arm at 5-m depth for the month of June are shown in Fig. 3. The spectra (Fig. 2 and 4) exhibit similar shapes except for the oxygen spectrum. Most of the variance comes from low frequencies or large scales. Log $f\Phi$ vs log f at smaller scales roughly follows a -2 slope corresponding to $\Phi \alpha f^{-3}$. If we consider the filter effect of the hose, then the slope at scales of about 10 m is steepened by approximately -1. Asymptotically the pump effect changes the slope by -2 at the smallest scales but here the system noise level is reached. Thus the -2 slope, when corrected, is really about -1 so that $\Phi \tilde{\alpha} f^{-2}$, corresponding to the results of Denman (1976). Salinity and temperature spectra are similar, but the temperature spectrum falls off slightly faster, possibly due to

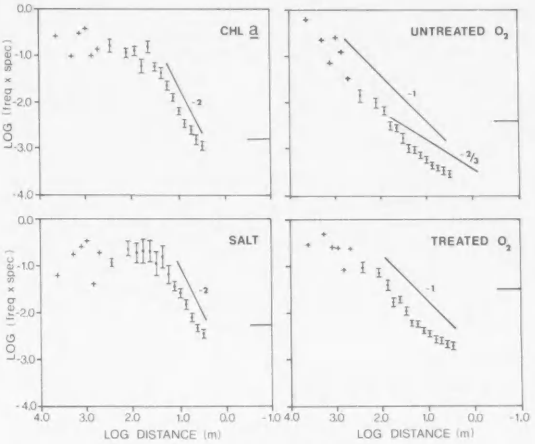

FIG. 4. Spectra of Indian Arm 5-m data for chlorophyll, oxygen, detrended oxygen, and salinity. Each spectrum has been normalized; to show absolute levels of spectra reference lines corresponding to $f\Phi$ values of 10^{-3} have been placed on each plot.

sensor limitations. Chlorophyll exhibits an even steeper slope, probably due to the limited response of the fluorometer.

The oxygen spectrum falls off with an $f\Phi\alpha - 1$ slope at the large scales and then levels off at the small scales because the system noise level has been reached. The large-scale results are likely caused by the large trend present in the original data (Fig. 3). This trend is larger than one would expect from a sensitivity decrease due to membrane aging and is probably real. However, because of the chosen sample length for analysis, energy in the trend has "leaked" and contaminated the spectrum of the fluctuations. Therefore, the oxygen data were detrended. The spectrum of the detrended data (Fig. 4) is much the same as the original in the mid- and small scales. The spectral shape of the larger scales is now similar to that of the other spectra.

In the midscale bands and small scales, the shape of the oxygen spectrum differs from the others. The original oxygen data (Fig. 3) do not appear to have as much activity in the midscales as the other parameters, which might reflect a problem with the probe. The −3 dB point for the oxygen probe may be at log distance ~2. The spectrum at smaller scales is then the result of instrumentation response limitation coupled with poor signal-to-noise conditions caused by having to use much of the dynamic range to record the trend.

For spectra other than that of oxygen the level of $f\Phi$ at large scales is just about constant and begins to decrease at about 50 m or so. The shape is similar to turbulent wind, temperature, and humidity spectra near the surface where those variables have flat $f\Phi$ spectra near the region of input of variance at about 20 times the height of observations and then drop off at higher frequencies (e.g. Miyake et al. 1970; Phelps and Pond 1971). In our case we also expect inputs at even larger scales associated with horizontal gradients.

Figure 5 shows the coherence spectra for the Indian Arm 5 m June data. Chlorophyll *a* is more coherent with temperature than with salinity. Oxygen and chlorophyll are the least coherent of the chlorophyll–physical parameter combinations. Temperature and salinity appear to be the most coherent variables except at the very small scales. The phase relationship between temperature and salinity is quite constant with salinity leading a little at the small scales as might be expected since the salinity sensor should have slightly faster response. The phases between oxygen and salinity and temperature show oxygen lagging in the midbands, further evidence of the limited response of the oxygen probe.

The results indicate a reasonably close relationship between physical and biological parameters suggesting that the distribution of chlorophyll, to a considerable extent, is determined by the dynamics of the inlet. The circulation would be expected to be dominated by the freshwater input from the Indian River at the head. The shear generated by the outflow of freshwater would be the source of most of the fluctuations and mixing. During the observations the winds were very light (mean speed $<2\ m \cdot s^{-1}$). Tidal mixing effects would be weak except perhaps near the sill away from the area of observations.

If there were no chlorophyll in the incoming river water, then a towed sampler would record patchiness simply because the saltwater contains plankton and the fresh does not, and an inphase relationship between salinity and chlorophyll would be observed. However, in the region where the data were collected, there would have been ample time for the chlorophyll to have been mixed and develop. In Indian Arm most of the production takes place in the top 5 m or so (Gilmartin 1960) and usually the vertical distribution will increase away from the surface, go through a maximum, and then decrease with depth. Data taken at a depth of 3 m indicate higher chlorophyll values than at 5 m. Therefore, in regions where there is stronger vertical mixing we might expect to find higher values of chlorophyll and lower values of salinity at 5 m. In general, the phase relationship between two parameters will be determined by the signs of their vertical gradients at the sampling depth.

Temperature and chlorophyll are 180° out of phase. The sign of the chlorophyll gradient suggests that temperature is increasing away from the surface of the inlet. This deduction seems reasonable since the freshwater input will be mainly the result of snow melt.

The plots of coherence involving oxygen and other parameters are all similar in shape. The observed coherence is fairly high, about 0.7, at the large scales and drops at log distance 1.2–1.5. This scale is about the same as that at which the oxygen spectrum (Fig. 4)

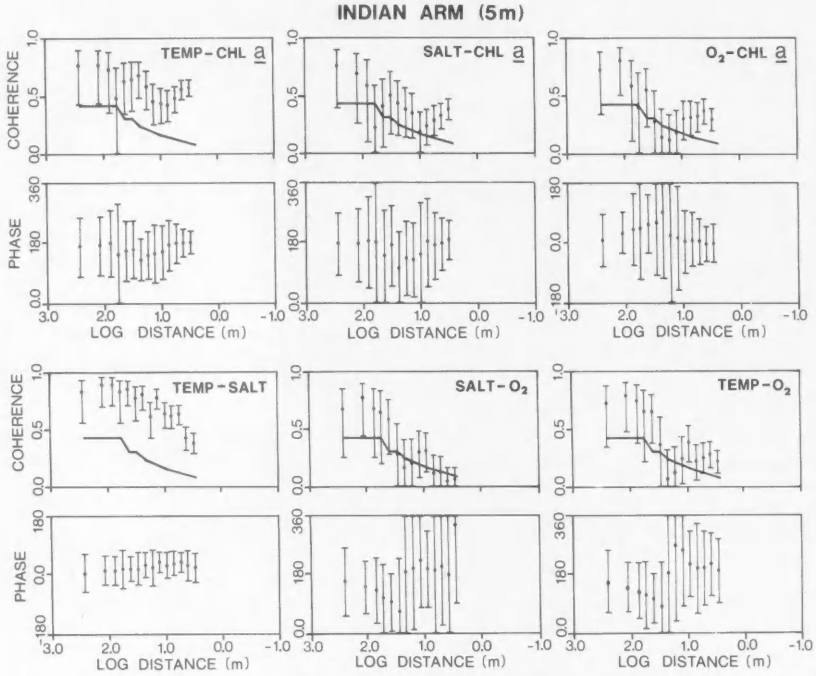

FIG. 5. Coherence and phase spectra for Indian Arm 5-m data.

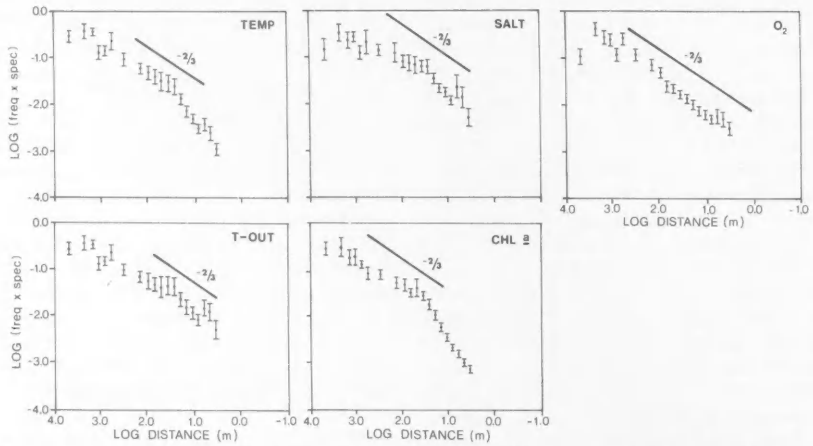

FIG. 6. Averaged normalized spectra for seven longer (10 or more blocks) runs. Averaging was done in frequency space and plotted against a distance scale calculated assuming $\bar{c} = 1.0\ \mathrm{m \cdot s^{-1}}$. Confidence interval is one of 68% for the mean and is based on observed variability.

changes to a slope of about −0.5 and most likely the oxygen system noise level is being approached.

AVERAGE RESULTS

Average spectral quantities (Fig. 6) and coherence and phase (Fig. 7) based on data from Indian Arm, Howe Sound, and the Strait of Georgia were calculated as described earlier. Although there were large variations from run to run, it was clear that on average there was a significant relationship between biological and physical parameters (Wiegand 1976).

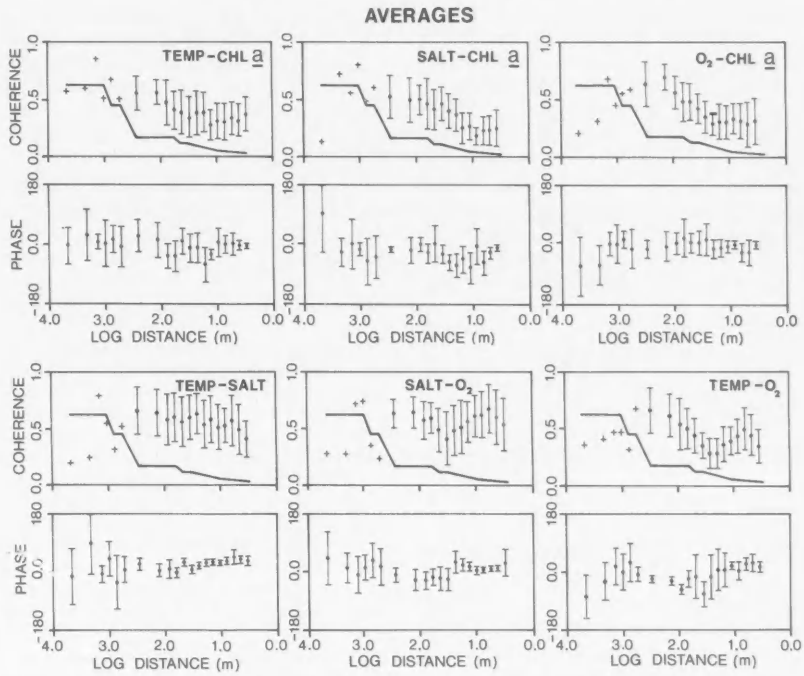

FIG. 7. Averaged coherence and phase for seven longer ($\geq$10 blocks) data runs. Confidence bands for coherence are 95% limits ($\pm$2 SE of the mean) based on observed variability in the data. The 95% significance level and phase confidence intervals were calculated from theoretical formulae (see text). Note that the phase has been forced to be near zero but for an individual run may be near zero or 180°. Coherence spectra from frequencies above the LS range, calculated from averages of the normalized co-spectra and quad-spectra values, were found to be essentially the same as those shown here.

Evidence of the smoothing action of the pump's mixing is found from the inboard (TEMP) spectrum which has a slightly steeper slope for log distance <1.3 than the outboard (T-OUT) spectrum. The average temperature spectra also show that most of the variance comes from large scales. The peaks near log distance $= 0.8$ are caused by ship motion during some runs in the Strait of Georgia. The slopes of both spectra are similar to a Kolmogoroff slope of $-\frac{2}{3}$ (Hinze 1959) except at small scales for TEMP because of the pump and at large scales where it is less steep where the Kolmogoroff prediction is not valid.

Chlorophyll shows a slightly smoother spectrum at the smaller scales partially because of instrument response and also because the variance due to ship motion was not present in the original data.

The average oxygen spectrum was calculated from detrended data and is indicative of the poorer signal to noise ratio of the probe, although down to about log distance $= 2.0$ it is similar to the other spectra.

The LS coherence results have only 14 df and there are peculiar phasing problems for some of the estimates. Thus, on average, the relations are poor although if enough data were available to sort them into groups having similar low frequency phasing, better coherences might result.

The confidence bands for coherence are similar to those of individual data runs although according to the theoretical formula they should be smaller because of the increased number of degrees of freedom. This sort of result is not uncommon in geophysical turbulence.

The average coherences are similar to those of individual runs already discussed. Both temperature and salinity seem fairly well related with chlorophyll at about the 0.5 level of coherence at the larger scales with the SALT-CHL A coherence maintaining this level down to log distance $\simeq 1.5$. This scale is associated with the "knee" in the salinity and chlorophyll average spectra and the drop in coherence for smaller scales might indicate that the source of variance for the parameters at this scale size are unrelated or that perhaps there is some cancellation in the co-spectrum because of phase differences. This drop in coherence is associated with a shift in the average phase toward 90° for this scale, indicating variability in the individual run phase estimates that went into the averaging. The

LS results for both TEMP-CHL A and SALT-CHL A are fairly high, about 0.6, but are only marginally statistically significant with only 14 df.

Oxygen appears fairly well related to chlorophyll between log distance = 3 and log distance = 1.5. Temperature shows coherences with oxygen that are similar to those of oxygen and chlorophyll except that the TEMP-O_2 results show a hint of an increase around the scale associated with ship motion (log distance $\simeq 0.8$) as does the salinity–oxygen coherence. For both TEMP-O_2 and SALT-O_2 the LS phases have wide confidence bands and coherences mainly below the significance level.

Temperature and salinity are related at about the 0.6 level down to scales of about log distance = 0.8, but in the LS range they do not appear to be significantly related. The phase estimates for the large scales show large error bars indicating an unsteady phase relationship which might possibly affect the coherence results.

Discussion

Spectral analysis of the data shows that the distribution of variance in the chlorophyll field as well as the other parameters is mainly at larger scales although occasionally there may be sources at smaller scales. The spectra are usually similar in shape suggesting that the sources of variance for the different parameters are similar. This hypothesis is further substantiated by coherence analysis (usually at the larger scales the coherence is greater than 0.5).

Cases existed where chlorophyll–temperature coherences were low yet chlorophyll–salinity coherences were high. Thus the coupling was not always expressed through all physical parameters (only in one of the longer runs when all three parameters had small variability were both chlorophyll–temperature coherences and chlorophyll–salinity coherences low). This result differs from Denman's (1976) where low chlorophyll–temperature coherences existed during runs having relatively large chlorophyll variance. In such cases the high chlorophyll variability was attributed to a fast-growing phytoplankton population. Our results may be an example of a different biological regime.

The relationships between parameters depend on the vertical nature of the distributions and vertical mixing (e.g. Indian Arm 5 m data). At larger scales horizontal gradients may also play a role. Sometimes there are peaks in the spectra at the smaller scales, <100 m or so; they may be caused by internal wave effects or ship motion.

The average spectra, allowing for the effects of pumping which are believed to steepen the slope by about -1 near 10 m, have slopes that are between 0 and -1 which corresponds to the results of other observers (e.g. Denman and Platt 1975; Powell et al. 1975) and are in agreement with recent theoretical work (Denman et al 1977), although this work is based on the assumption of a homogeneous and isotropic turbulent velocity field which is probably not justifiable for our data.

In summary, there appears to be a measurable relationship between the biological and physical worlds, much of the time, especially at larger scales. Turbulent mixing processes appear to affect the distribution of phytoplankton.

Profiling fairly rapidly, as well as moving horizontally, would allow better resolution of both horizontal and vertical variations and is recommended for future investigations.

Acknowledgments

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Distribution of Nutrients in the Gulf of St. Lawrence

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COOTE, A. R., AND P. A. YEATS. 1979. Distribution of nutrients in the Gulf of St. Lawrence. J. Fish. Res. Board Can. 36: 122–131.

The general pattern of nutrient distribution in the Gulf of St. Lawrence results from regeneration processes being superimposed on the physical processes of estuarine circulation within the Gulf. This leads to a general increase in nutrient concentration with depth and with distance into the Gulf from Cabot Strait. Nutrient concentrations in the Laurentian Channel are higher inside the Gulf than at equal or even greater depths in the Atlantic Ocean some distance outside Cabot Strait. Summer nutrient concentrations in the surface layer are generally low. However, during the winter when biological activity is low, quite high concentrations of the nutrients are found in the surface waters of the Gulf. A balance exists between inward and outward fluxes of all three nutrients through Cabot Strait in the winter. However, in the summer the influxes of both nitrate and silicate at Cabot Strait greatly exceed the outgoing fluxes. Losses of biogenic silica to the sediments may account for the silicate imbalance. Excess nitrate may be accounted for if ammonia or nitrogen bound with dissolved organics had been measured.

Key words: nutrients, silicate, phosphate, nitrate, distribution, regeneration, transport, Gulf of St. Lawrence, Cabot Strait, Laurentian Channel

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La configuration générale de la distribution des éléments nutritifs dans le golfe Saint-Laurent résulte de processus de régénération qui se superposent sur les processus physiques de circulation estuarienne à l'intérieur du Golfe. Ceci entraîne une augmentation générale des éléments nutritifs en fonction de la profondeur et de la distance dans le Golfe depuis le détroit de Cabot. La concentration des éléments nutritifs dans le chenal Laurentien est plus élevée à l'intérieur du Golfe qu'à la même profondeur ou une profondeur plus grande dans l'océan Atlantique à une certaine distance du détroit de Cabot. Les concentrations estivales des éléments nutritifs dans la couche superficielle sont généralement faibles. Cependant, en hiver, quand l'activité biologique est ralentie, on trouve des concentrations assez élevées d'éléments nutritifs dans les eaux superficielles du Golfe. Il existe un équilibre entre l'afflux et l'efflux des trois éléments nutritifs dans le détroit de Cabot en hiver. Cependant, en été, l'afflux à la fois des nitrates et des silicates dans le détroit de Cabot dépasse de beaucoup les efflux. On peut expliquer le déséquilibre des silicates par les pertes de silice biogénique dans les sédiments. On peut expliquer l'excès de nitrates si l'on mesure l'ammoniaque ou l'azote liées aux matières organiques dissoutes.

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THE Gulf of St. Lawrence is an important fisheries region in Canada and, because of the role nutrient salts play in primary production, an understanding of the distribution, circulation, and chemistry of these compounds is important. The only extensive study of the silicate, nitrate, and phosphate concentrations in the water of the Gulf of St. Lawrence was the McGill IBP study (Bulleid and Steven 1972; Steven et al. 1973; Steven 1974) which emphasized the upper 50 m of the water column. Data on the nutrient concentrations in the deeper water of the Gulf, particularly on concentrations during winter, are less abundant. In this study, we have tried to fill these data gaps and to relate the nutrient concentrations to water circulation and regeneration.

The Gulf of St. Lawrence (Fig. 1) is a marginal sea with an area of 250 000 km^2 connected to the Atlantic Ocean through Cabot Strait and the Strait of Belle Isle. Cabot Strait with a width of 104 km, a maximum depth of 480 m, and a cross-sectional area of 10^6 m^2 is the principal connection to the Atlantic. The St. Lawrence River with a mean annual discharge of 10^4 m^3/s contributes approximately two thirds of the river input to the Gulf (Trites 1972). The physical oceanography of

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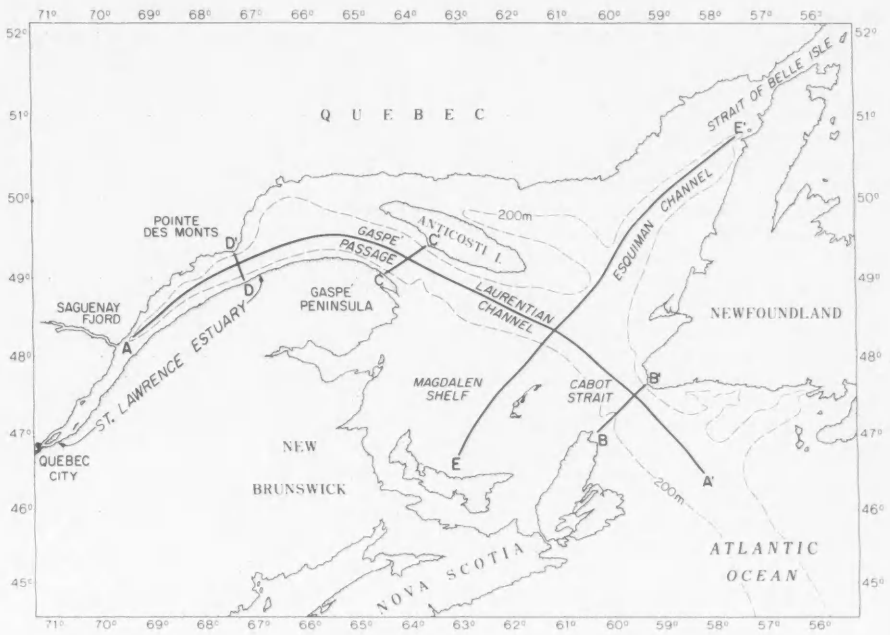

FIG. 1. Gulf of St. Lawrence including locations of major nutrient sections. Station positions are given in Coote and Hiltz (1975).

the Gulf of St. Lawrence has been reviewed by Trites (1972), who describes the circulation pattern as a two-layer system, with surface water flowing out from the St. Lawrence River predominantly on the southeastern side of the Gulf and a deep layer flowing much more sluggishly from Cabot Strait into the estuary. Salinities in this deep layer are relatively constant at ~34.5‰.

Methodology

The concentrations in the water column of the three primary nutrient salts, silicate [$Si(OH)_4$], nitrate ($NO^{2-} + NO^{3-}$), and phosphate (predominantly HPO_4^{2-}) were measured at a number of stations during three cruises over a 2½-yr period. These were Bedford Institute (BI) Cruise 71-027, July 12–August 8, 1971, on the C.F.A.V. *Sackville*, BI Cruise 72-017, May 30–June 13, 1972, on the C.S.S. *Dawson*, and BI Cruise 73-004, February 12–March 8, 1973, on the C.S.S. *Hudson*. The complete listing of these nutrient data and salinity and temperature data are reported by Coote and Hiltz (1975).

On all three cruises nutrient samples were collected at three to eight depths from the surface to near the bottom in Knudsen or Niskin bottles and analyzed for the nutrients on board ship using a Technicon AutoAnalyzer. All samples were unfiltered and were poisoned after collection with 3–5 drops of chloroform and stored at 4°C before analysis. Silicate and nitrate were measured using the automated methods of Strickland and Parsons (1968) and phosphate by the method of Atlas et al. (1971), using hydrazine sulfate as the reducing agent. Concentrations are all expressed in microgram-atoms per litre. The precision and accuracy of silicate and phosphate results using the AutoAnalyzer I have been described by Coote et al. (1971) for their *Hudson*-70 data; CSK nitrate standards were not then available.

Results

The two summer cruises showed similar distributions of the three nutrients in all areas of the Gulf except at the mouth of the St. Lawrence River where higher concentrations of silicate, phosphate, and nitrate were observed in the surface water during 1972. High surface concentrations were also observed in the seaward-flowing current along the Gaspé coast. Otherwise, surface concentrations were generally low during these two cruises particularly in the northern half of the Gulf. Similar surface distributions were observed in the McGill study (Bulleid and Steven 1972; Steven et al. 1973; Steven 1974). In February 1973 surface concentrations were much higher than during the summer cruises, decreasing from a maximum of 19.9 μg-atoms/L Si, 1.3 μg-atoms/L P, and 14.5 μg-atoms/L N in the St. Lawrence Estuary to ~4 μg-atoms/L Si, 0.7 μg-atoms/L P, and 6 μg-atoms/L N in Cabot Strait. The entire northern region of the Gulf has similar surface concentrations to those found in Cabot Strait.

For the two summer cruises, the concentrations of silicate, nitrate, and phosphate increased with depth

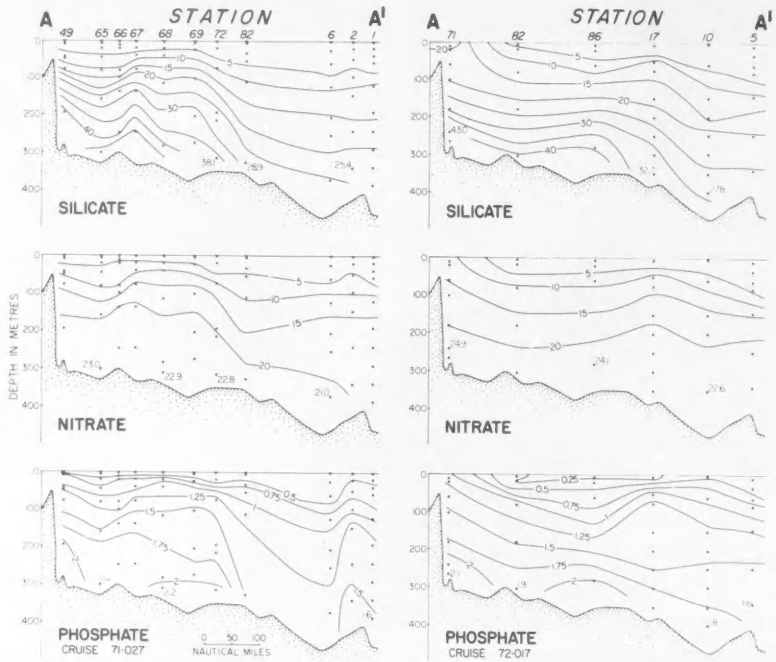

FIG. 2. Summer nutrient distributions in the Laurentian Channel (section A–A′ in Fig. 1). Concentrations are in microgram-atoms per litre.

from the surface to the bottom and with distance from Cabot Strait to the St. Lawrence Estuary (Fig. 2). This general increase with depth is found everywhere in the Gulf except the Saguenay Fjord. Silicate varies from <1 μg-atoms/L to 25 μg-atoms/L from the surface to near bottom in Cabot Strait. In the deep layer silicate increases from ~25 μg-atoms/L in Cabot Strait to ~40 μg-atoms/L in the lower estuary. Phosphate and nitrate also show increased concentrations with depth and from Cabot Strait to the estuary, but the increases in the deep layer are smaller. Phosphate increases from ~0.3 to 1.6 μg-atoms/L with depth in Cabot Strait and from 1.6 to 2.1 μg-atoms/L in the deep layer from Cabot Strait to the estuary. Nitrate increases from 0.3 to ~22 μg-atoms/L with depth and to ~24 μg-atoms/L at depth in the estuary. In February 1973 (Fig. 3) the deep-layer distributions were similar to the summer distributions, but surface concentrations of all three nutrients were higher.

Three sections across the Laurentian Channel were occupied on all three cruises (Fig. 1, BB′, CC′, and DD′). At Cabot Strait (Fig. 4 and 5) there was no concentration gradient across the Strait in summer (cruises 71-027 and 72-017) for any of the three nutrients. However, for 73-004, concentrations of all three in the upper 200 m were higher in the southern than the northern half of the Strait. This region of higher concentration is generally associated with the outflowing water from the Gulf of St. Lawrence. At Gaspé Passage (Fig. 6) and Pointe des Monts (Fig. 7) high nutrient concentrations were found in the near-surface water on the southern sides of the two sections. Again, these parts of the sections contain the outflowing water from the St. Lawrence Estuary. At Pointe des Monts there is also evidence of nutrient enrichment in the deep water on the northern side of the estuary. Concentrations at these stations, and on Cruise 71-027 at stations to the northeast of Pointe des Monts, are approximately 20% higher than at adjacent stations in the Laurentian Channel. Evidence for a gyre in this region has been advanced by El-Sabh (1975).

The Esquiman Channel, the major deep trough in the northern part of the Gulf, showed a similar distribution of nutrients with depth to that in the Laurentian Channel. The concentrations in the deep water at the southern end of the Esquiman Channel were comparable to concentrations at similar depths in the adjacent area of the Laurentian Channel. However, between the Laurentian Channel and the head of the Esquiman Channel silicate increased noticeably on cruise 71-027 (Fig. 8). There was also evidence of smaller increases in both nitrate and phosphate.

Relatively low nutrient concentrations were found on the Magdalen Shelf although the concentrations in the subsurface water on the shelf may be somewhat higher than at similar depths in adjacent areas.

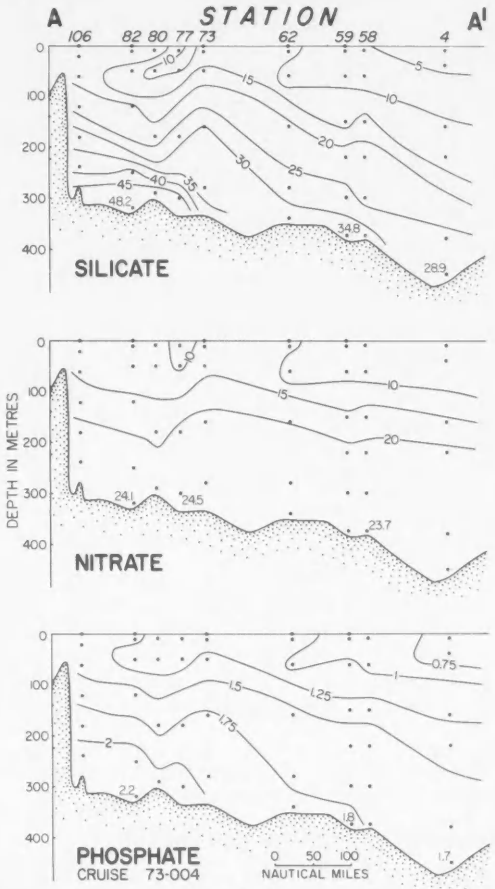

FIG. 3. Winter nutrient distributions in the Laurentian Channel (section A–A′ in Fig. 1). Concentrations are in microgram-atoms per litre.

The Saguenay Fjord is the only region where the nutrient concentrations did not increase with depth (Coote and Hiltz 1975). Silicate was highest at the surface, 20–30 μg-atoms/L, and decreased with depth to 100 m. Nitrate increased from the surface to 30 m, where concentrations were approximately 10 μg-atoms/L, and decreased from there to 100 m. Phosphate was fairly uniform vertically at ~0.3 μg-atoms/L. No samples were collected in the fjord at depths greater than 100 m.

Discussion

DISTRIBUTION

Concentrations of nutrients in the deep layer are all approximately 3 times as high as concentrations at similar depths in the North Atlantic outside the Gulf (A. R. Coote unpublished data), indicating that the Gulf of St. Lawrence is a trap for nutrients. This enrichment is a result of a combination of water circulation and nutrient regeneration processes. Subsurface water with high nutrient concentrations flows inward (i.e. upstream); is mixed into the surface layer by entrainment with St. Lawrence River water, upwelling, or other mixing processes; and flows out toward Cabot Strait in the surface layer. Plankton in the surface layer use these nutrients, die, and some sink back into the intermediate layer where the nutrients are regenerated and returned to the surface farther upstream. In this way, there is a cycling of nutrients within the Gulf between the intermediate layer and the surface effectively trapping nutrients. The upwelling or entrainment of nutrients in the St. Lawrence Estuary is of particular importance as a major source of nutrients to the surface waters of the Gulf. If regulation of freshwater run-off from the St. Lawrence River resulting in decreased discharge in summer and increased discharge in winter decreases entrainment in the estuary in summer and increases entrainment in winter, as suggested by Neu (1975), not only will the amounts of nutrient reaching the surface layer during spring and summer be reduced, but the ability of the Gulf to trap nutrients may also be decreased. This reduction will occur because increased winter circulation will cause large quantities of nutrients to be lost from the Gulf when the internal cycling mechanism is attenuated due to the absence of biological activity.

This estuarine circulation process has also been used to explain both the slow inward movement of deep water and the increasing deep water nutrient concentrations toward the head of the Laurentian Channel. However, a similar concentration increase, which occurs towards the north end of the Esquiman Channel, cannot be due to estuarine circulation because of the absence of significant freshwater inputs at the northern end of the channel. It may well be that in neither case is the very deep water significantly affected by estuarine processes.

A plot of nutrient concentrations vs. depth at one station (Fig. 9) illustrates: (1) the year-to-year consistency in the concentration, particularly for nitrate and phosphate, at any depth below 150 m; (2) the high surface concentrations in Cruise 73004 because diminished phytoplankton growth in the colder surface water during winter allows upwelled nutrients or nutrients released by dying plankton to remain in the surface layer; and (3) the difference in the profiles for nitrate and phosphate on one hand and silicate on the other; i.e. silicate concentration increases steadily to the bottom whereas nitrate and phosphate remain virtually constant in the deepest water. This suggests that most of the nitrate and phosphate is regenerated in the upper part of the water column, which is consistent with the evidence that the sediments of the Gulf are low in organic matter (Loring and Nota 1973). Silicate dissolution, however, seems to continue to the bottom.

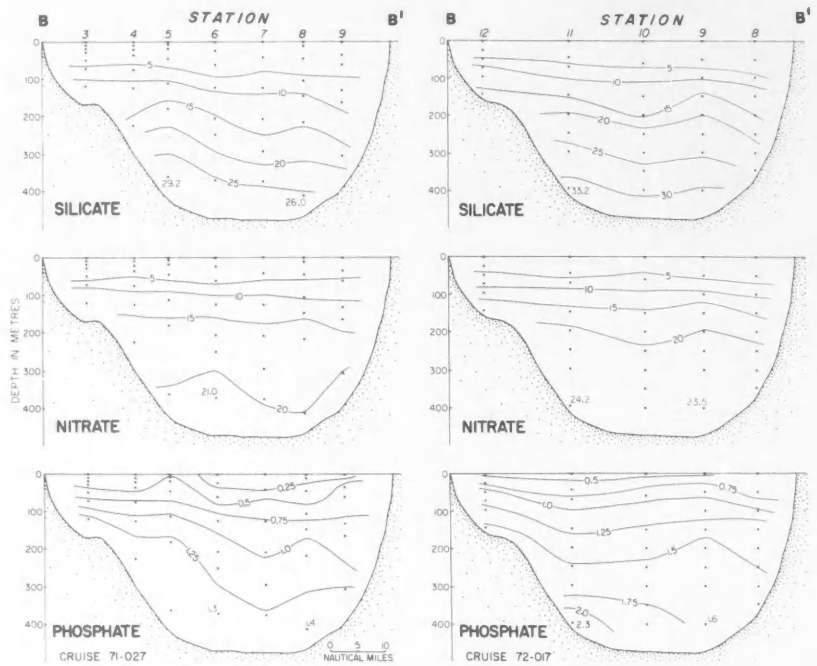

FIG. 4. Summer nutrient distributions at Cabot Strait (section B–B′ in Fig. 1). Concentrations are in microgram-atoms per litre.

NUTRIENT REGENERATION

Nitrate to phosphate ratios of 16:1 have been obtained consistently in subsurface water outside the continental shelves. In coastal regions a larger range has been observed usually with considerably lower N:P ratios. The N:P ratio will be determined by the relative utilization and release of nitrate and phosphate by microorganisms, N:P ratios of mixing water masses, and external sources of nitrate and phosphate such as rivers. The linear regressions of phosphate on nitrate derived in this study by combining all the nutrient data for each cruise are

$$N(\mu\text{g-atoms/L}) = 10.3\ P(\mu\text{g-atoms/L})\text{-}0.7 \qquad n = 417,\ r = 0.56$$

$$N(\mu\text{g-atoms/L}) = 15.3\ P(\mu\text{g-atoms/L})\text{-}5.5 \qquad n = 213,\ r = 0.88$$

$$N(\mu\text{g-atoms/L}) = 14.6\ P(\mu\text{g-atoms/L})\text{-}5.7 \qquad n = 166,\ r = 0.80$$

for cruises 71-027, 72-017, and 73-004, respectively. The N:P ratios are in good agreement with the commonly quoted value of 16:1 (Redfield et al. 1963) except for cruise 71-027 which has a high proportion of near surface samples. If these regressions are recalculated for all samples on stations deeper than 200 m in order to remove the overabundance of nearsurface data, the following results are obtained:

$$N(\mu\text{g-atoms/L}) = 14.5\ P(\mu\text{g-atoms/L})\text{-}3.4 \qquad n = 117,\ r = 0.87$$

$$N(\mu\text{g-atoms/L}) = 15.4\ P(\mu\text{g-atoms/L})\text{-}5.0 \qquad n = 104,\ r = 0.88$$

$$N(\mu\text{g-atoms/L}) = 15.5\ P(\mu\text{g-atoms/L})\text{-}5.7 \qquad n = 108,\ r = 0.90.$$

Only the result for cruise 71-027 is changed appreciably. The cruise to cruise consistency is improved and the N:P ratio for 71-027 is in better agreement with the value of Redfield et al.

During summer, biological activity reduces the concentrations of all three nutrients in the top 50 m of the water column. Nitrate seems to be the limiting nutrient, and in most of the Gulf the nitrate concentration is <0.2 μg-atoms/L in the upper 20 m. Phosphate is less depleted with the result that the N:P ratio is often reduced to 1 or less. From the regressions of P on N, the phosphate concentration corresponding to [N] = 0 is ~0.3 μg-atoms/L. In February 1973, surface depletion was not observed, and surface nitrate and phosphate concentrations fitted closely to the regression line.

The linear regressions referred to above are derived

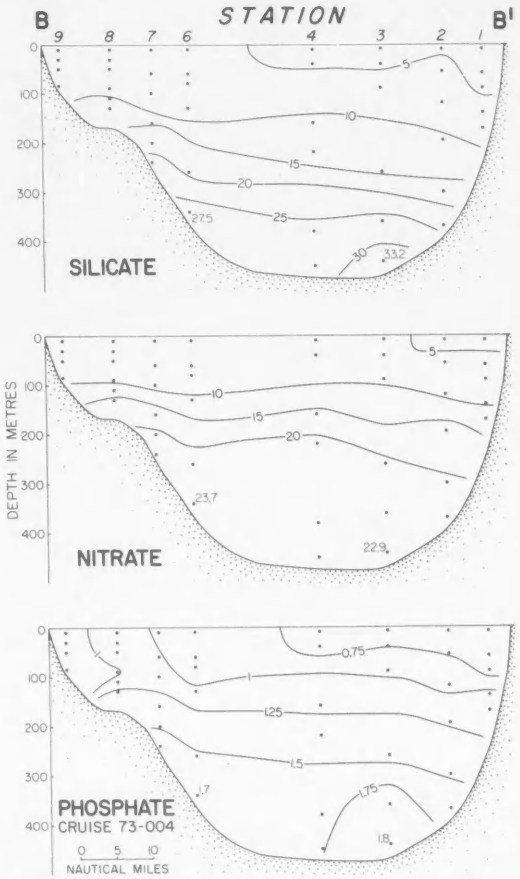

FIG. 5. Winter nutrient distributions at Cabot Strait (section B–B′ in Fig. 1). Concentrations are in microgram-atoms per litre.

from large data sets, including data from surface water, water entering through Cabot Strait and the deep water of the troughs. However, the nature of the circulation pattern and the year-to-year consistency in nutrient and dissolved oxygen concentrations at any location in the deep inflowing layer of the Laurentian Channel allowed us to relate changes in the nutrient and oxygen concentrations along the Channel to regeneration of nutrients solely in this deep layer. In doing this, we assumed that little or no water from other sources was being incorporated into this layer, and the absence of change in the salinity with distance at any particular depth would support this assumption. As the water flowed in the deep layer from Cabot Strait to the estuary, the concentrations of silicate, phosphate, and nitrate increased and the oxygen concentration decreased due to decomposition of organic matter which sinks into this layer. For example, the nitrate concentration in the deep layer increased from Cabot Strait to the estuary (Fig. 10). The same trend was found for silicate and phosphate and the opposite trend for oxygen. The net changes in the concentrations of silicate, phosphate, nitrate, and oxygen at 250 m depth were 30 μg-atoms/L, 5.6 μg-atoms/L, 0.80 μg-atoms/L, and −2.5 mL/L, respectively, on cruise 71-027 and 25, 3.5, 0.50, and −1.7, respectively, on cruise 72-017. These concentration changes corresponded to ΔO:ΔN:ΔP ratios of 278:7.0:1 and 298:7.2:1 for nutrients regenerated in the deep layer from Cabot Strait to the estuary. The ΔO:ΔP ratios were near the accepted values of 276:1 (Redfield et al. 1963) but the ΔN:ΔP ratios, although virtually identical for both cruises, were somewhat lower than the ideal oceanic value of 16:1. However, lower ΔN:ΔP ratios in coastal and estuarine environments are not unusual (Ketchum et al. 1958; Sen Gupta and Koroleff 1973). The low ΔN:ΔP ratio may indicate that nitrate regeneration is less complete than phosphate regeneration or the organic matter being regenerated is simply low in nitrogen. The ratio of ΔSi:ΔP from this analysis is about 40:1.

NUTRIENT BUDGETS

No nutrient budget has previously been constructed for the Gulf of St. Lawrence. While considerable nutrient data have been accumulated recently, certain types of pertinent data are still unavailable. For example, little experimental data exist for atmospheric interactions or for rates of sedimentation in the Gulf. In the case of transport calculations, the work of Forrester and El-Sabh (1974) indicates that sectional data gathered over a tidal cycle are desirable. Total nitrogen and phosphorus data are not available. This is obviously a serious limitation as ammonia and dissolved and particulate organic nitrogen may form a significant fraction of the total nitrogen and phosphorus concentrations. Nevertheless, a crude budget calculation, making use of the data available and some reasonable estimates for processes and concentrations not yet examined in the Gulf, should provide some useful information regarding nutrient fluxes and help to define inadequacies in the data.

Because the nutrient concentrations in the surface layer vary greatly between summer and winter, separate nutrient fluxes through Cabot Strait have been calculated for summer (8 mo between approximately April and November with low surface concentrations) and winter (4 mo December–March with high surface concentrations) (Table 1). These estimates have been made using the water transport data of Trites (1972) for summer and El-Sabh and Johannessen (1972) for winter. The nutrient data used for Cabot Strait are shown in Fig. 4 and 5. In the summer calculation, the core of the inflowing water is assumed to be at 100 m, intermediate between the two extremes suggested by Trites (1972). The nutrient transports were calculated from

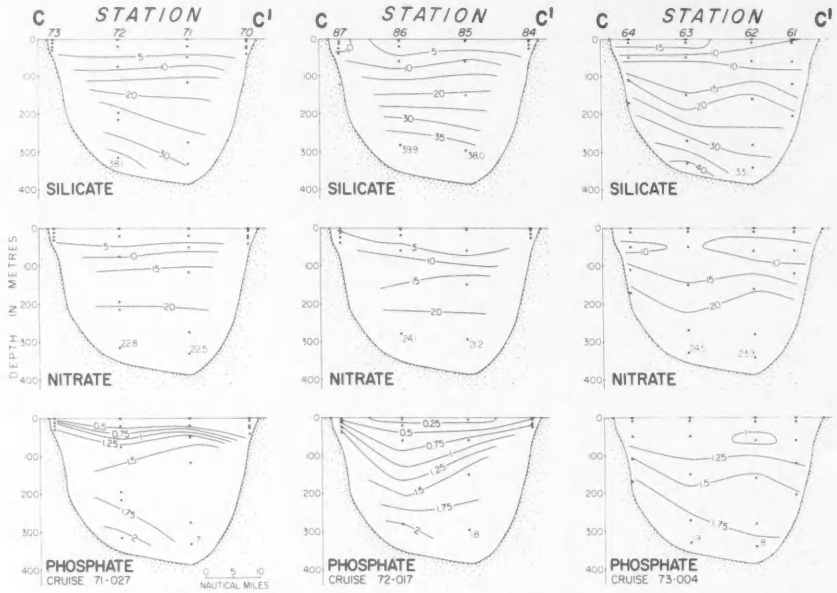

FIG. 6. Nutrient distribution at Gaspé Passage (section C–C′ in Fig. 1). Concentrations are in microgram-atoms per litre.

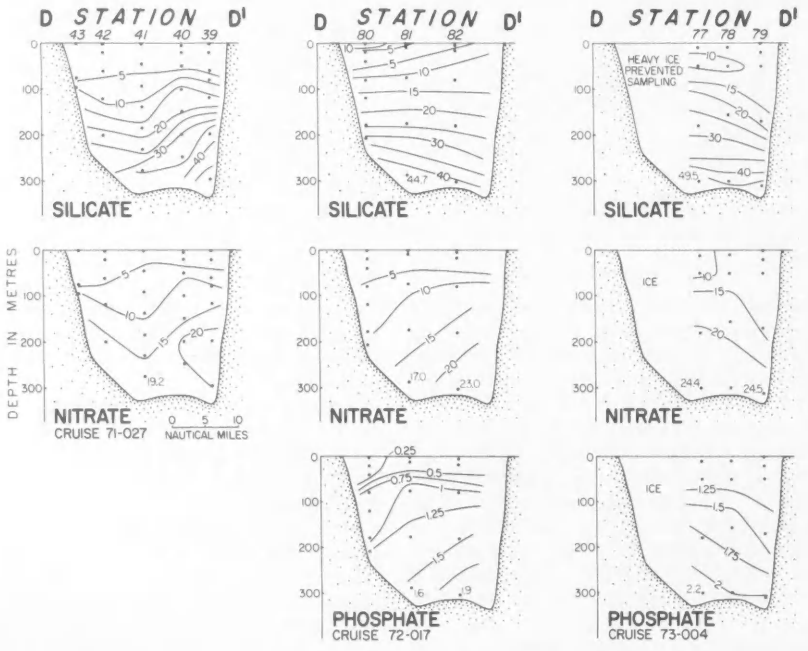

FIG. 7. Nutrient distribution at Pointe des Monts (section D–D′ in Fig. 1). Concentrations are in microgram-atoms per litre.

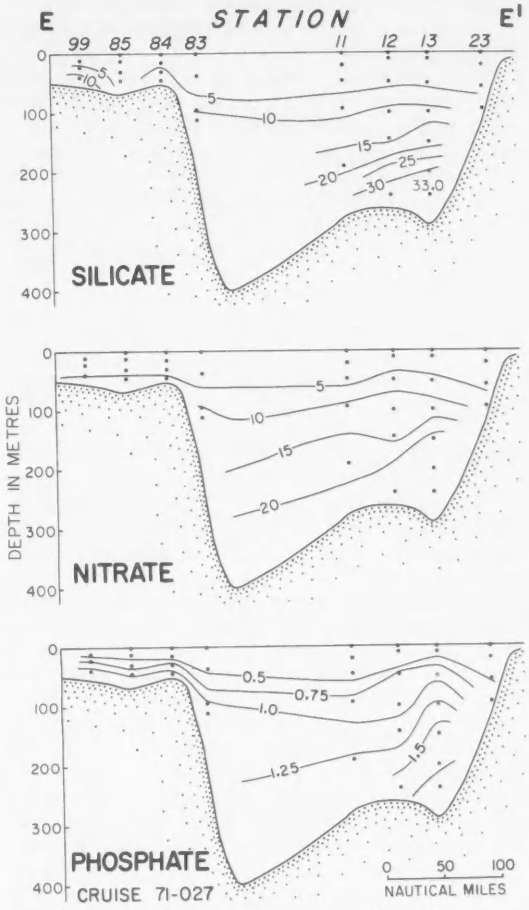

FIG. 8. Nutrient distribution in the Esquiman Channel and Magdalen Shelf (section E–E' in Fig. 1). Concentrations are in microgram-atoms per litre.

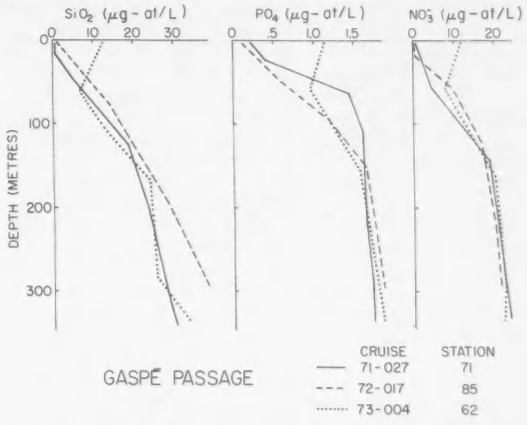

FIG. 9. Yearly variation in nutrient vs. depth profiles at Gaspé Passage.

these data by plotting the nutrient and water flow sections on a grid, and summing the product of water transports times nutrient concentrations for both the inflowing and outflowing water masses.

River inputs were calculated using average values for the St. Lawrence River nutrient concentrations of Si = 32, P = 1.5, and NO_3 = 14 μg-atoms/L at Quebec City (NAQUADAT 1973) and a total river flow of 1.3×10^7 L/s (Trites 1972). The resulting fluxes are 3.8×10^5 t/a* for silicate, 2.1×10^4 t/a for phosphate, and 8.4×10^4 t/a for nitrate. Rainfall based on Junge's (1963) data for North America could add about 3.5×10^4 t/a of oxidized nitrogen compounds. Rainfall concentrations for phosphate and silicate are unavailable but expected to be small. The total atmospheric input of solids based on an areal share of global fallout is 4×10^4 t/a (Sundby 1974), so that contributions from this source would also be small compared to the flows through Cabot Strait.

Suspended particulate matter enters the Gulf via rivers and through Cabot Strait, but about 3 times as much leaves the Gulf through Cabot Strait as enters (Sundby 1974). Maximum losses by this route, assuming all the suspended matter is of biological origin with a C:Si:N:P ratio of 105:20:16:1, are 7×10^5 t/a silicate, 3.7×10^4 t/a phosphate, and 5.2×10^5 t/a nitrate. The silicate loss would be greater if more of the suspended matter were SiO_2 derived from diatom skeletons, but on the other hand would be less if the concentrations of inorganic detrital material were significant. The losses of phosphate and nitrate to the sediment in organic matter are 4.3×10^3 t/a, and 1.3×10^4 t/a, respectively, based on a 2% average organic content (Loring and Nota 1973) and sedimentation rate of 1.3×10^3 g/m² per year (Sundby 1974). Losses of biogenic silica to the sediments are harder to estimate. Loss due to evaporation of ammonia and nitrous oxide is also possible. A theoretical flux of nitrous oxide suf-

TABLE 1. Dissolved nutrient fluxes in millions of tonnes per year calculated for Cabot Strait.

	Summer		Winter	
	Inflow	Outflow	Inflow	Outflow
Silicate	5.8	3.9	2.6	3.0
Nitrate	3.4	2:2	1.2	1.3
Phosphate	0.49	0.45	0.37	0.40

*SI abbreviation for year as recommended by the National Standard of Canada Canadian Metric Practice Guide Can3-Z234.1-76.

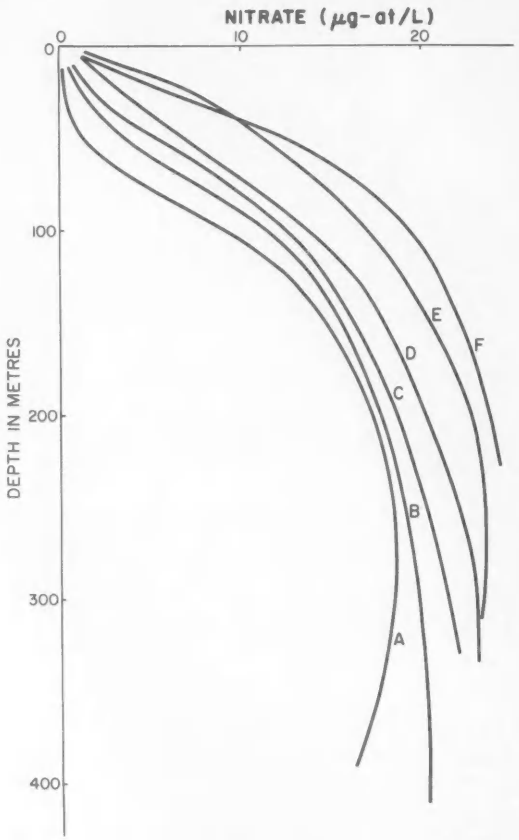

FIG. 10. Changes in the nitrate vs. depth profile with distance landward in the Laurentian Channel on Cruise 71-027. Profile A is to the east of Cabot Strait, B is at Cabot Strait, C is in the central Gulf, D is at Gaspé Passage, E is at Pointe des Monts, and F is in the St. Lawrence Estuary.

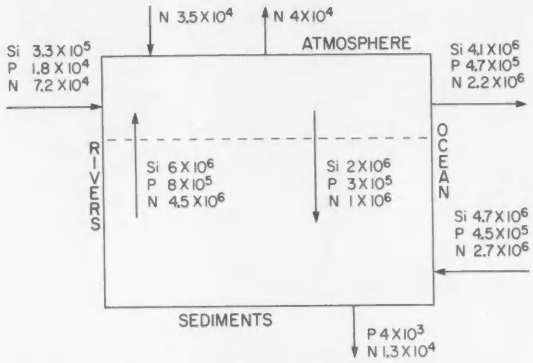

FIG. 11. Nutrient budget for the Gulf of St. Lawrence (tonnes per year).

ficient to account for the global nitrogen budget imbalance has been calculated (Liss and Slater 1974) and on this basis 4×10^4 t/a could evaporate from the surface of the Gulf of St. Lawrence. The combined fluxes of silicate, phosphate, and nitrate from all these sources and sinks are shown in Fig. 11.

In winter the budget is essentially in balance for all three nutrients. In summer, however, both silicate and nitrate appear to be significantly out of balance. The Gulf appears to be receiving an excess of 1.6×10^6 t/a of silicate and 0.8×10^6 t/a of nitrate. Calculation of the internal fluxes of silicate between the surface and deep layers in the Gulf, based on estimates of the amount of silicate introduced to the surface layer from the deep layer by mixing and the amount returned to the deep layer during the regeneration process, indicates that the major losses of silicate from the water column occur in the deep layer. The most likely sink for the 1.0×10^6 t/a imbalance is biogenic silicate debris settling to the sediments. Settling and dissolution of particulate silicate apparently occurs all the way to the sediments, as indicated by the dissolved silicate profile (Fig. 9). If sufficient silicate to balance the budget were being incorporated into the sediments, silicate would account for ~20% of the sedimentation in the Gulf of St. Lawrence, if Sundby's (1974) estimate for sedimentation rate is valid. Sundby's estimate, however, is intended to be conservative, and larger sedimentation rates may be possible. In the budget calculated for suspended matter in the Gulf of St. Lawrence (Sundby 1974), an internal source of suspended matter of about 5×10^6 t/a is needed to explain the sedimentation rates in the Gulf. Formation and subsequent sedimentation of silicate tests may possibly account for both the source of suspended matter and the sink for silicate. Undetected polysilicate in the outflowing water, and errors in the estimate of silicate loss in the suspended particulate matter may also make important contributions to the silicate balance.

The nitrate case is more complicated because not only are there several different important nitrogen-containing species, but some of these are volatile or important components of atmospheric precipitation. For the Gulf of St. Lawrence, the outflow of nitrate is only three quarters of the total input. Estimates of the internal fluxes between the two main water masses indicate that the discrepancy occurs predominantly in the surface layer. The imbalance of 6×10^5 t/a could possibly have been accounted for if dissolved organic nitrogen, ammonia, or other nitrogen-containing species were measured, as it seems likely that these compounds would have higher concentrations in the shallower outflowing water than in the deeper inflowing water. A concentration difference of approximately 4 μg-atoms/L would be needed for a balance. There is an obvious need for more dissolved organic nitrogen and ammonia measurements in this system.

In the calculation of the dissolved nutrient fluxes through Cabot Strait 100 m was chosen as the depth for the core of inflowing water. Geostrophic calculations by El-Sabh (1975) indicate that 75 m may be a better choice. If we use this depth the phosphate influx would be reduced by ~5% and the silicate and nitrate by ~10%. These changes would account for less than half the silicate and nitrate imbalances and would also cause an imbalance in the phosphate budget of $\sim 3 \times 10^4$ t/a. An additional source of phosphate would then be needed to balance the phosphate budget.

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Estimated Escapement of Atlantic Salmon (*Salmo salar*) for Maximum Smolt Production in Rivers of Different Productivity

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SYMONS, P. E. K. 1979. Estimated escapement of Atlantic salmon (*Salmo salar*) for maximum smolt production in rivers of different productivity. J. Fish. Res. Board Can. 36: 132–140.

Smolt production at different egg deposition densities is estimated from data on survival rates and space requirements of juvenile Atlantic salmon (*Salmo salar*) reported in the literature. Average maximum production of smolts is estimated to be approximately 5/100 m² for 2+ smolts, 2/100 m² for 3+ smolts, and 1/100 m² for 4+ smolts. Minimum egg depositions recommended for production of these numbers of smolts are 220/100 m², 165–220/100 m², and 80/100 m² for each age of smolts, respectively. The escapement of adults required to produce these depositions must be estimated from observed average weights of returning females and a reported fecundity of Atlantic salmon between 1650 and 1760 eggs/kg of female. With the exception of Ungava rivers, average smolt age in any particular river can be estimated from the number of days per year on which water temperature reaches or exceeds 7°C.

Key words: fishery resources, fishery management, production (biological), escapement, survival, game fish, freshwater fish, rivers

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Nous fondant sur les données contenues dans la documentation publiée sur les taux de survie et les exigences spatiales de jeunes saumons atlantiques, nous avons estimé la production de smolts à différentes densités de dépôt d'oeufs. On estime que la production maximale moyenne de smolts est d'environ 5/100 m² pour les smolts de 2+, 2/100 m² pour ceux de 3+ et 1/100 m² pour ceux de 4+. Les dépôts d'oeufs minima recommandés pour la production de ces nombres de smolts sont 220/100 m², 165–220/100 m² et 80/100 m² pour chaque âge de smolts respectivement. Le nombre d'adultes qui doivent s'échapper pour produire ces dépôts doivent être estimés à partir des poids moyens observés des femelles qui retournent en rivière et d'une fécondité du saumon atlantique variant entre 1650 et 1760 oeufs/kg de femelle. Sauf pour les rivières de l'Ungava, l'âge moyen des smolts dans une rivière quelconque peut être estimé à partir du nombre de jours par an où les températures de l'eau atteignent ou dépassent 7°C.

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ELSON (Elson and Tuomi 1975) estimated escapement requirements for maximum equilibrium yield of Atlantic salmon (*Salmo salar*) to the river Foyle, Ireland, through development of a model (Ricker 1954) based on an optimal egg deposition of 168 eggs/100 m² (=150 eggs/100 yd²) of total area of the river. A review of the literature on production of juvenile Atlantic salmon in streams (P. E. K. Symons unpublished data), led to estimates of egg deposition requirements that in the case of rivers producing 2-yr-old smolts were higher than those suggested by Elson for the river Foyle. These new estimates also suggested that egg deposition should vary depending on age of smolts produced from the river and other factors. Although these egg depositions and resultant escapement requirements were based on a much less sophisticated analysis than that of Elson, they offered an alternative guide for management, which was perhaps more easily and widely applicable to different river systems.

The overall objective of this new analysis is to estimate the carrying capacity of a river for juvenile salmon. Carrying capacity is estimated from three sets of data: survival rates from egg to smolt; growth rates to smolt stage (smolt ages); and space requirements of young salmon at all stages between fry and smolt. From this estimate of carrying capacity, maximum smolt production and the number of eggs and females required to produce this number of smolts are estimated. Sur-

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vival and growth of Atlantic salmon in the sea between smolt and returning adult stages are of minor concern in this paper.

Data Base

Survival From Egg to Smolt

Survival rates vary from location to location and from year to year depending on numerous environmental factors such as temperature, degree of sedimentation in the stream, streambed movement, predation, etc. However, within any naturally maintained salmon population there are lower limits below which survival cannot fall without threat of population extinction. Also, while the upper limit to survival is 100%, it is unlikely to be this high except over a very short time (a few days). There is, therefore, a range of survival rates through the life stages (egg, fry, parr, and smolt) of Atlantic salmon within which any naturally maintained population is likely to remain. The immediate objective is to define this range.

Survival of Atlantic salmon from egg-to-emergent fry has been studied by planting "green" ova in slatted perspex boxes buried to a depth of 50 cm in the gravel of the River Dee, Scotland (Shearer 1961). Survival was estimated after hatching by subtracting the number of dead eggs and alevins remaining in the boxes from the known number of eggs buried. Survival ranged from 85 to 91%.

Elson (1962a, 1975) has provided data relating density of young fish to survival between egg and smolt (Table 1). Egg densities in the Pollett River, New Brunswick, were derived from counts of adults passing upstream through a fish counting fence. Smolts (88% 2-yr olds, 12% 3-yr olds) were counted moving downstream through the same fence. Annual population estimates of fry and parr in the river were made from repeated seine fishing of pools and riffles, sampling up to 9% of total rearing area. Population estimates of yearling or older juveniles were probably reliable within 20% (Elson 1962a), but estimates of underyearlings were too low since their survival to age 1 was estimated to exceed 100% in 4 of 8 yr. Survival estimates that utilize underyearlings have been omitted from Table 1.

Additional data from the same river are available for plantings of hatchery-reared underyearling salmon (Elson 1975) which were made over a number of years, with and without the control of fish-eating birds (mergansers, *Merganser americanus*, and kingfishers, *Megaceryle alcyon*). Here the exact number of underyearlings is known, but survival of hatchery fish is likely to be lower than that of naturally produced salmon of the same age (Elson 1975).

In the Pollett River from 1942 to 1960 average survival from egg to smolt of naturally produced salmon ranged between 0.8% at high egg densities (725 eggs/100 m²) and 3.8% at low egg densities (34–88 eggs/100 m²). Inspection of survival rates from yearling juveniles to smolts emigrating mostly a year later suggested that survival at this stage was generally 40–50%. Wider variations at medium egg-deposition densities may have arisen in part from errors in the estimates of yearlings. Survival of yearlings to smolts was apparently little affected by egg density, meaning compensatory mortality must have occurred earlier.

Survival of hatchery-reared underyearlings to 1+ parr demonstrated this compensatory relation with density. Survival decreased in years with bird control from a high of 26% at low underyearling densities (9/100 m²) through 17% at medium densities (70/100 m²) to a low of 8% at high densities (256/100 m²). The same pattern was apparent without control of predatory birds, but survival was on average 7% lower. There were no plantings at the highest density. Survival from yearling to smolt stage again appeared to be independent of density and was generally more than twice that of fry to yearlings. This pattern of density-dependent mortality in the post-hatching or larval stages of fish, with adjustments at later stages occurring through regulation of body growth, was first noted by Ricker (1954).

TABLE 1. Survival (%) between various stages of juvenile Atlantic salmon calculated from data of Elson (1975) for the Pollett River. Where data for more than 1 yr have been averaged, the range and number of years of data are given in parentheses.

Natural production	Virtual[a] egg deposition (number/100 m²) Low 45 (34–88, 4 yr)	Medium 186 (152–228, 3 yr)	High 725
Egg to 1+ parr	9.1 (5.8–16.3)	3.4 (2.8–4.2)	2.3
1+ parr to 2+ smolt	44 (38–50)	51 (14–81)	40
Egg to 2+ smolt	3.8 (2.6–6.2)	1.6 (0.6–2.7)	0.8

Hatchery production	Density of planted hatchery-reared underyearlings (number/100 m²) Low 9 (5–18, 3 yr)	Medium 69 (65–76, 5 yr)	High 256
Without control of predatory birds			
Underyearling to 1+ parr	<25 (<25, 2 yr)	5	—
1+ parr to smolt	>35 (>20–>50)	43	—
Underyearling to smolt	9 (6–12)	2	—
With control of predatory birds			
Underyearling to 1+ parr	26	17 (9–23, 4 yr)	8
1+ parr to smolt	49	50 (29–79)	34
Underyearling to smolt	12	8 (6–9)	3

[a]Virtual egg deposition = 75% potential egg deposition given in Elson's Table 2, using Elson's suggested adjustment for loss of spawners to angling and poaching.

Data on survival from egg to 3+ smolts are given for Atlantic salmon in the northwest Miramichi River by Paloheimo and Elson (1974). Numbers of ascending adults and descending smolts were counted at a fence, though in most years this fence could not be installed sufficiently early in the spring to trap the entire smolt run, and in 5 yr of the 21 yr for which there are data a large proportion of the run was probably missed. Juvenile salmon populations, estimated from electrofishing only 0.2% of total stream area (2.5×10^6 m² derived from Elson 1967 and Elson 1975), were unreliable since survival of juveniles (0+ to 1+ and 1+ to 2+) exceeded 100% in 40% of the cases. However, overall survival from egg to 3+ smolt (for smolt ages see Forsythe 1967 and 1968) at different egg densities (Table 2) are useful for comparison with egg-to-smolt survival in streams producing primarily 2+ smolts (Table 1). In the Miramichi, survival to 3+ smolt was about half that of 2+ smolts at comparable egg densities in the Pollett.

Meister (1962) provides data on survival from egg to juveniles of age 0+ to 3+ in Cove brook, Maine. Egg deposition was estimated from the sex and weight of adults ascending past a counting fence. Juvenile populations were estimated by electrofishing and mark–recapture techniques. Prior to calculating survival, I subtracted the number of juveniles (1+ to 3+) emigrating from the stream past the

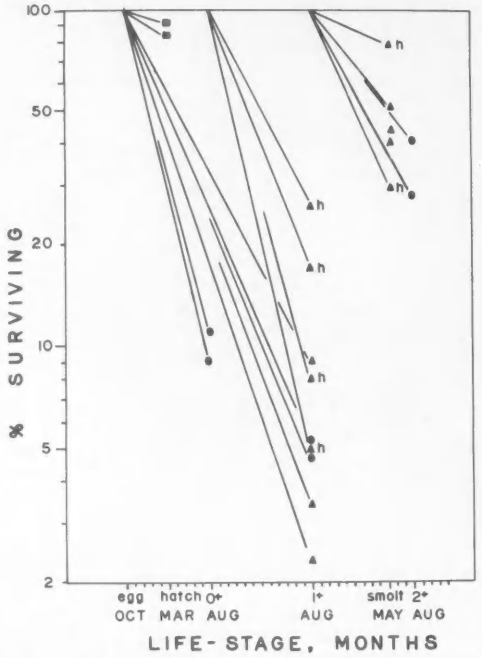

FIG. 1. Survival of juvenile Atlantic salmon (*Salmo salar*) between various life stages summarized from the literature: ■, Shearer (1961); ●, Meister (1962); ▲, Elson (1975); "h" denotes planted hatchery-reared fish; other data are for naturally produced salmon.

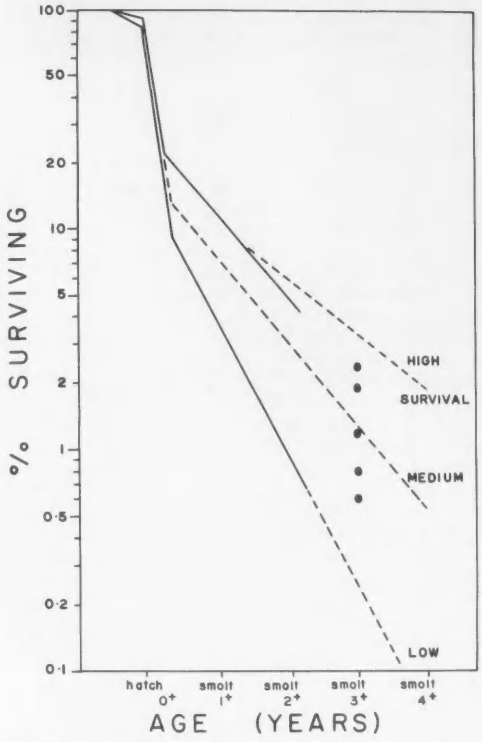

FIG. 2. Range of survival through the life-stages of juvenile Atlantic salmon estimated from Fig. 1 by connecting successive low or high survival rates of naturally produced fish at various stages (—). Survival beyond 2+ has been extrapolated, high survival being adjusted upward on the basis of observed survival of hatchery-reared juveniles between age 1+ and 2+ (- - - - -). Medium survival rates drawn between low and high rates by eye. Survival to 3+ smolt (●) from Table 2.

fence from these population estimates. This was not done by Meister, and the survival rates plotted in Fig. 1 therefore differ slightly from those in Meister's table.

Gee et al. (1978) studied mortality of juvenile Atlantic salmon in the river Wye, Wales. Fry and parr populations were estimated at 16 sites, 3–6 times over a 1–2-yr period. Despite the authors' assertion that loss of fish from individual sites could be equated with mortality, some losses, particularly those at high density downstream sites, probably resulted from emigration. Alevins, fry, and parr of Atlantic salmon may move downstream in the fall (Saunders 1972; Thorpe and Morgan 1978). This may be followed by upstream movement in the spring and summer (Huntsman 1945). If upstream movement occurred chiefly along the main watercourse into the headwaters, movement alone could have accounted for the higher losses in the nine stations downstream from the junction of the Marteg tributary, compared with those in the seven headwater stations (with the exception of one headwater station too shallow to retain many 1+ or older salmon). Because of this possible confusion of movement with mortality, these data are not included.

All apparently reliable data on survival of naturally produced Atlantic salmon are summarized in Fig. 1, together with some information on hatchery salmon planted by Elson (from Table 1). These data have been used to provide estimated ranges of survival through successive stages from egg to smolt (Fig. 2). These estimates have been smoothed on the assumption that after hatching survival increases with each year of life to the smolt stage. Resultant survival rates only partially agree with those of Elson (1962b). Elson used population estimates of underyearlings in his calculations that were rejected in the present work because they appeared to be too low. Consequently, his survival estimates from egg to underyearling are lower, and from underyearling to yearling are higher than those in Fig. 2. Elson's estimates of survival of older fish (1+ to 2+ and 2+ to 3+) agree well with those given here.

Because no data are available on survival of juveniles from age 2 yr to 3 yr or older, survival of juveniles older than two has been estimated by extrapolation. To obtain theoretically minimal and maximal rates, I assumed either the lowest observed rates followed one another in successive stages, or the highest rates did. Under natural conditions, low survival at one stage might more usually be followed by medium to high survival at the next. At present there is no way of knowing whether the extremes estimated occur in practice. However, survival from egg to age 3+ smolt in the northwest Miramichi River (Table 2) brackets the

TABLE 2. Smolt production and survival from egg to age 3+ smolt in the Northwest Miramichi River (data from Paloheimo and Elson 1974).

Virtual egg deposition			
Range	Avg (no. yr)	Avg no. smolt/100 m²	% Survival eggs to smolt
31–33	32 (2)	0.6	1.9
57–66	62 (2)	1.5	2.4
75–87	81 (2)	1.0	1.2
100–130	120 (4)	0.9	0.8
148–200	176 (6)	1.0	0.6

estimated medium rate in Fig. 2, and the observed range covers at least half that estimated. Estimated survival rates derived from Fig. 2 which are to be used in later calculations are summarized in Table 3A.

GROWTH, SMOLT-AGE, AND SPACE REQUIREMENTS

Atlantic salmon smolts may range in age from primarily 1+ in the warm fertile rivers and streams of southern England and France to 7 or 8 yr of age in rivers of northern Norway, and of Ungava Bay in northern Quebec (Templeman 1967). Smolts from almost all rivers were between 12 and 18 cm long (Elson 1957), the large (19–22 cm) smolts of Ungava rivers being exceptions (Power 1969). Because of similar lengths of smolts in different rivers, the average smolt age in a particular river can be used as an index of rate of growth of juvenile salmon in that river.

Growth of juvenile Atlantic salmon in streams, as measured by length increments, is approximately linear with each year of age (Symons 1970). Annual length increments of salmon in rivers with smolts age 2+ to 4+ are reviewed by Symons (1970). With additional data from Frost and Went (1940), a summary can be prepared (Table 3B) giving approximate average lengths at midsummer for each age-class of juvenile salmon in streams producing smolts aged 1+ to 4+.

The space requirements of young salmonids of various lengths were reviewed by Allen (1969). Allen concluded that there was a positive linear relation between average area required by individual fish and fish length, when both

TABLE 3. Data used to estimate smolt production.

A.

Survival rates	Eggs to 0+	Annual juvenile survival	Juvenile to smolt	Eggs to smolt: Smolts 1+	2+	3+	4+
Low survival	0.09	0.28	0.35	0.032	0.009	0.003	<0.001
Medium survival	0.13	0.41	0.52	0.070	0.028	0.012	0.005
High survival	0.20	0.44 (0+ to 1+)	0.55 (1+ smolt)	0.110	0.057	0.032	0.019
		0.57 (1+ or older)	0.65 (2+ to 4+ smolt)				

B. Approximate lengths and space utilized by individual young salmon[a] of these lengths at three population densities in midsummer (Aug. 1).

	Age 0+	1+	2+	3+	
To produce:					
1+ smolt	9 cm				(Length from Frost and Went 1940)
Low pop.	10.5 m²				
Average pop.	5.5 m²				
High pop.	2.0 m²				
2+ smolt	6 cm	12 cm			(Lengths from Symons 1970)
Low	5.0 m²	40 m²			
Average	1.5 m²	10 m²			
High	0.5 m²	5 m²			
3+ smolt	5.5 cm	8.5 cm	11.5 cm		
Low	4.5 m²	10 m²	40 m²		
Average	1.0 m²	5 m²	9 m²		
High	0.5 m²	1 m²	4.5 m²		
4+ smolt	5 cm	7.5 cm	9.5 cm	12 cm	
Low	4 m²	8 m²	20 m²	40 m²	
Average	0.8 m²	4 m²	6.5 m²	10 m²	
High	0.4 m²	0.8 m²	2.5 m²	5 m²	

[a]"Space utilized" is not equatable with "year-class density" observed in the field unless areas utilized by other year-classes in the same habitat are first subtracted from the total area fished.

TABLE 4. Matrix of estimated production of 2+ smolts/100 m² at three rates of survival and three population densities (from Table 3), estimated proportion of total rearing area required for underyearlings (nursery), and egg deposition/100 m².

Populations	Survival		
	Low	Medium	High
Low	0.6 smolts 30% nursery 68 eggs	1.0 smolts 24% nursery 36 eggs	1.3 smolts 23% nursery 23 eggs
Medium	2.3 smolts 35% nursery 261 eggs	3.8 smolts 27% nursery 136 eggs	4.9 smolts 26% nursery 86 eggs
High	5.2 smolts 26% nursery 591 eggs	8.4 smolts 20% nursery 300 eggs	10.2 smolts 18% nursery 179 eggs

were plotted on logarithmic scales. Additionally, nearly all observed areas per fish fell within a factor of three of the average for that length. From Allen's figure, the space requirements of all age-classes of juvenile Atlantic salmon at their respective lengths in midsummer can be estimated for rivers producing smolts aged 1+ to 4+. This can be done for low, medium, and high population densities, spanning the observed range of densities described by Allen (1969) (Table 3B).

SYNTHESIS OF SURVIVAL RATES AND SPACE REQUIREMENTS

Using the data on survival rates and space requirements, smolt production can be estimated for streams where various growth rates, juvenile population densities, and survival rates exist. The numbers of eggs and juveniles required to produce one smolt are estimated from the low, intermediate, and high survival rates in Table 3A. The total space requirements for each age-class of juveniles are estimated as the product of the number of juveniles of each age times their individual space requirements depending on their length (Table 3B) summed over the age-classes present. Estimated annual smolt production (expressed as smolts per 100 m²) = 100 m²/(Σ space requirements (m²) for all stages to produce one smolt). The egg deposition/100 m² required for this annual production of smolts is estimated from the product of annual smolt production/100 m² times number of eggs required to produce one smolt.

Four matrices of annual smolt production and egg deposition were generated, one for each of four ages of smolts, for three rates of survival v. three levels of population density. Because underyearlings <7 cm have different habitat requirements from larger juveniles (Symons and Heland 1978), and such "nursery" habitats might limit numbers of underyearlings independently of the space available for older juveniles, the percentage of rearing ground required as a "nursery" has also been included. The matrix generated for 2+ smolts is given as an example (Table 4). Data from the Pollett River (Table 1) suggest that as population density of juvenile salmon increases from low through medium to high, survival decreases from high through medium to low. Therefore smolt production was plotted against egg deposition (Fig. 3) using the inverse densities and survival rates from the matrices.

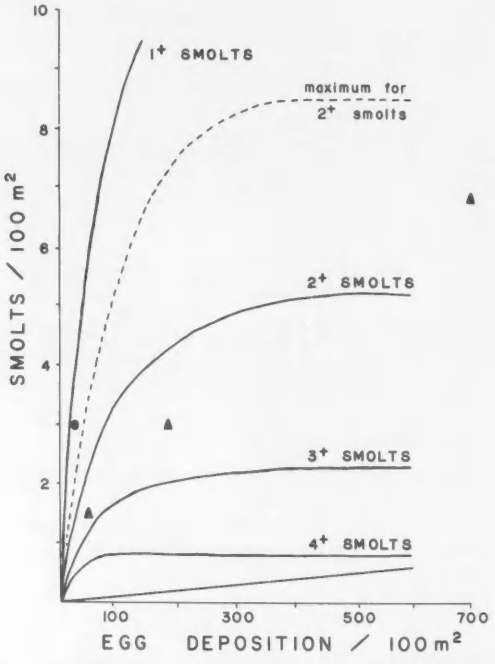

FIG. 3. Estimated average production curves (see text) at varying densities of egg deposition for smolts aged 1+ to 4+ (—), and maximum potential production curves for 2+ smolts (- - - - -). Observed production of 2+ smolts from Meister 1962 (●) and Elson 1975 (▲). Bottom diagonal line gives minimal smolt production at different egg deposition densities below which the population would not be selfsustaining (see text).

The resulting production lines for 2+, 3+, and 4+ smolts (Fig. 3) give the average number of smolts that might be expected at various levels of egg deposition in good salmon producing streams (like the Pollett, from which the curves were partially derived). A maximum theoretical limit is suggested for 2+ smolts in Fig. 3 by plotting high survival against medium population densities, and medium survival against high population densities, and then by assuming an approximately parallel curve to that derived for average conditions. This potential maximum might be reached under extremely favorable weather or stream conditions. Similarly estimated potential maxima for 3+ and 4+ smolts fall approximately on the average smolt production curves for smolts 1 yr younger, respectively, and therefore no separate lines have been drawn.

There are also lower limits to production. These arise when survival to the smolt stage is so low that after mortality at sea too few adults return to maintain the population. Elson (1975) suggests that a large (2-sea-years or more) female salmon weighing 3.6 kg would produce about 6300 eggs. Each spawning female, to maintain the stock, must produce on average two offspring that return to spawn, or, assuming a maximum (and very high) survival of 30% between smolt and adult (Saunders and Allen 1967) at

least six smolts. The ratio of smolt production to egg production must therefore exceed 6:6300 or 0.001 if the population is to be self-sustaining. This lower limit to production is also shown in Fig. 3.

Predicted Smolt Production and Escapement Requirements

The smolt production curves suggest that average production of smolts varies about tenfold (~1–~10 smolts/100 m^2) depending on the age of smolts produced. Where smolts are all one age, the difference from stream to stream or year to year between average and maximum production estimates is greater for 2+ and 3+ smolts (5–8 or 2–5 smolts/100 m^2) than for 4+ smolts (0.8–2.3 smolts/100 m^2). Potential maximum production of 1+ smolts exceeds 20/100 m^2, but the medium survival and high juvenile densities required to produce this number may not be attainable in the warm fertile streams producing 1+ smolts. A potential maximum equal to the average maximum of approximately 10 smolts/100 m^2 at medium survival and juvenile densities may be more realistic.

The effect of egg deposition on smolt production also varies with the age of smolts. For 4+ smolts maximum production is attained with an egg deposition of approximately 75 eggs/100 m^2 or greater; 3+ smolts require an egg deposition of approximately 150–200/100 m^2 to reach maximum production; 2+ smolts require still higher levels of egg deposition, although a deposition of 200/100 m^2 under average conditions should produce approximately 85% (4.5 smolts/100 m^2) of the average maximum (5.2 smolts/100 m^2). Given the variability expected and observed (see Table 1, and observed production points in Fig. 3), this difference would in practice probably not be detectable except with data from many years. To produce 10, 1+ smolts/100 m^2, an egg deposition of at least 140 eggs/100 m^2 is estimated to be required, but potential production of 1+ smolts continues to increase with egg depositions up to 500/100 m^2.

The sharp inflection and flattened top of the production curve derived for 4+ smolts become successively less pronounced as smolt age decreases. This suggests that, provided a minimum escapement at the point of inflection is exceeded, changes in fishing pressure and in escapement will have less effect on subsequent smolt production and commercial yield in regions where the catch is supported by rivers producing 4+ smolts, than where the fishery depends on rivers producing younger smolts.

Escapement requirements can be estimated on the basis of egg deposition requirements and the fecundity of Atlantic salmon. Fecundity has been cited as approximately 1650 eggs/kg of female (Meister 1962, quoting various earlier references) or 1760 eggs/kg of female (Baum and Meister 1971). Because the average weight of females varies among rivers, and may vary within a river in different years depending on angling and commercial fishing pressure, feeding, growth in the sea, and other factors, the escapement required to meet management objectives must be estimated separately for each river and for each year or period of years.

Estimated midsummer nursery requirements for production of smolts under the normal inversely related levels of survival and juvenile population densities vary maximally between 17 and 43% of total juvenile rearing ground. The higher proportions of nursery-to-total rearing ground are required when survival rates are low and juvenile population densities high. Nursery requirements for production of 2+ to 4+ smolts under medium levels of survival and population density range between 18 and 27% of total juvenile salmon habitat. Nursery requirements for production of 1+ smolts cannot be estimated because by midsummer most underyearlings have surpassed the 7-cm length at which movement to deeper, faster water has commenced (Symons and Heland 1978). If nursery grounds make up 25% of total rearing grounds, requirements under most normal conditions would apparently be met.

Critiques and Refinements

Smolt Production at Optimum and Above Optimum Egg Deposition

Both Elson's analysis and the present one predict that production of 2+ smolts will decrease rapidly as egg deposition decreases below 170 eggs/100 m^2, but they differ widely in their prediction of smolt production when egg deposition increases above 170 eggs/100 m^2. The present analysis suggests that maximum smolt production is maintained, indeed to some extent ensured, by higher egg deposition, whereas Elson's analysis suggests production rapidly declines once an "optimal" deposition of 170 eggs/100 m^2 is surpassed. Since this difference might alter management strategy concerning allowance of an excess escapement as a margin of safety or margin for error, the reasons for the difference are worth examining.

Elson's model is based on a conclusion that there is an interaction between yearling or older salmon and underyearlings such that when numbers of yearlings are high, the survival of underyearlings is low. His conclusion also requires that yearlings reduce numbers of underyearlings so severely that later, after the older fish have departed as smolts, the underyearling year-class cannot recover significantly, despite the above-average survival expected at their low density. Elson (1975) himself recognized the limitations of the data supporting this conclusion, and stated that further investigation was warranted.

Gee et al. (1978) present a curve with an optimal fry density for maximum production of presmolts which might be construed to support Elson's conclusion. However, these authors' optimal fry density of 75/100 m^2 requires a minimal egg deposition of 375/100 m^2, assuming a maximum egg-to-fry survival of 20% (Table

3A). This egg density is 2.2 times the optimum recommended by Elson. Still higher but suboptimal fry densities of Gee et al. would require egg depositions mostly in excess of 600/100 m², assuming an average egg-to-fry survival of 13%. These exceed the maximum considered here for whole rivers. Such high egg densities may occur over restricted areas, but then emigration of fry would almost certainly exceed immigration, invalidating the mortality rates from which the optimal fry density curve was derived.

In the absence of direct evidence for postulating optimum egg and juvenile densities above which smolt production is reduced, alternative assumptions have been made. These are that juvenile densities and smolt production reach maxima that are dependent on size of juvenile and age of smolt, respectively, and that there is no depression in smolt production at high egg densities to at least 600/100 m². These assumptions are compatible with the principle of density-dependent mortality, particularly with the dependence of underyearling mortality on the density of their own and older age-classes. Below a maximum total density of all year-classes, equivalent to the carrying capacity of the river, mortality increases slower than density, so that the density of each year-class remains positively correlated with its egg deposition. Above this maximum, underyearling mortality becomes sufficient to impose a ceiling on underyearling numbers, and therefore also on the density of that year-class at later stages.

Allen (1968) suggested that the maximum density of any species of stream salmonid was roughly 170 g/100 m², and that nearly all densities fell within a factor of three of this value, providing a potential maximum of 510 g/100 m². There are at least two references to higher biomass densities: maxima of 1060 g brook trout (*Salvelinus fontinalis*)/100 m² (Shetter and Leonard 1942), and 590–1290 g brown trout (*Salmo trutta*)/100 m² in 6 of 10 streams examined by LeCren (1969). Maximum densities of juveniles used to estimate maximum smolt production may, therefore, have been conservative, at least for periods <1 yr.

Rejection of the hypothesis of reduced smolt production at egg depositions above an optimum would favor a management strategy that provides an excess egg deposition to provide a margin for errors in estimates of escapement, weights of females, fecundity, rearing area available, etc. If an arbitrary 10% margin for error is allowed, an optimum escapement with least wastage of spawners would aim to produce an egg deposition of at least 220 eggs/100 m² for production of 2+ smolts, between 165 and 220 eggs/100 m² for production of 3+ smolts, and a minimum of 80 eggs/100 m² for 4+ smolts. While Elson's recommended egg deposition of 170 eggs/100 m² appears sufficient for the Miramichi River which produces mostly 3+ smolts, these new estimates suggest that an increase of 30% (to 220 eggs/100 m²) is necessary for the Foyle River which produces primarily 2+ smolts.

Smolt Age, Temperature, and Latitude

Because smolt age is a significant feature in estimates of required escapement and egg deposition for maximum smolt production, the factors controlling smolt age are worthy of brief review.

Growth is directly related to food intake which in turn is determined by food availability and the vigor with which the fish feeds (Allen 1969). Production of fish and their invertebrate food organisms is to some extent positively correlated with calcium content of the water (LeCren 1972; Mann 1971). Growth of poikilotherms is also temperature dependent, and Allen (1941) pointed out a trend for older smolts to be produced at higher latitudes.

Temperature is certainly important to growth and production of Atlantic salmon. Power (1969) suggested that a summer with at least 100 d at a mean temperature of 6°C or higher was a minimal requirement. At temperatures below 7°C juvenile Atlantic salmon move from riffles to pools and reduce or cease feeding (Allen 1940; Saunders and Henderson 1969). Digestion of food by brown and rainbow trout (*S. gairdneri*) at temperatures below 7.3°C is too slow to permit more than one full meal per day (Elliott 1973). Above 7°C the growth rate of Atlantic salmon fry increases, reaching a maximum at 16.6°C (Siginevich 1967). Little is ap-

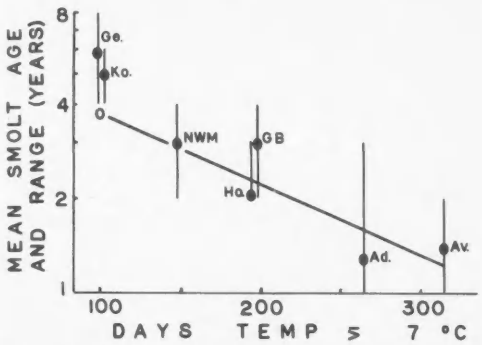

FIG. 4. Mean smolt age (●), and range in ages (|) at various lengths of growing season defined as days per year in which water temperatures reached or exceeded 7°C. Age of Ungava salmon 15 cm long, the usual length of smolts from other rivers (○). Data for Ungava rivers (George River, Ge., and Koksoak River, Ko.), Canada, from Power (1969); for northwest Miramichi River (NWM), Canada, smolt-ages Forsythe (1967, 1968); river temperatures P. F. Elson, St. Andrews, N.B., Canada, personal communication; for Høgvädsen River (Ho.), Sweden, G. Edman, Falkenberg, personal communication; for Girnock burn (GB), Scotland, R. J. G. Buck, Agriculture and Fisheries, Aberdeenshire, personal communication; for River Adour (Ad.), France, smolt age, Vibert (1950), river temperatures, M. Heland, Station d'Hydrobiologie, Biarritz, personal communication; for Avon River, England, A. S. Grater, Avon and Dorset River authority, personal communication.

parently known of the growth of this species at temperatures above 16°C.

Because growth of juvenile Atlantic salmon at temperatures below 7°C is slow (Allen 1969), the growing season in a river may be defined as the number of days per year in which the water temperature reaches or exceeds 7°C. Approximately 500 d at or above 7°C are required to produce a 15-cm smolt. In southern rivers these 500 d are accumulated in <2 yr, whereas in Ungava rivers 4 yr are needed for young salmon to reach 15 cm (Fig. 4). Because Ungava salmon smoltify at 19–23 cm (Power 1969), they require an additional 1 or 2 yr of stream residence to become smolts. Figure 4, though an oversimplification of the relation between temperature and growth, is a useful index for estimating smolt age in salmon streams where temperature records are available, but data on smolts are not.

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Differences in the Age of Pacific Hake (*Merluccius productus*) Using Whole Otoliths and Sections of Otoliths

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BEAMISH, R. J. 1979. Differences in the age of Pacific hake (*Merluccius productus*) using whole otoliths and sections of otoliths. J. Fish. Res. Board Can. 36: 141–151.

Ages determined for older Pacific hake from sections through the nucleus of otoliths were older than the ages determined from the otolith surface for the two populations studied. Growth of all parts of the otolith was not identical throughout the life of the fish. The change in the pattern of otolith growth occurred about the age at which the growth in length of the fish was reduced. Despite the disproportionate growth in length and height of the otolith, the thickness of the otolith, especially in the ventral interior portion, continued to increase with age. Section ages appear to be more reliable for older fish because of this allometric change in otolith growth with increasing fish age. It is suggested that the examination of sections of otoliths should become a routine approach in any attempt to age fish using otoliths.

Key words: age determination, otolith, otolith section, otolith growth, aging errors, Pacific hake, Strait of Georgia

BEAMISH, R. J. 1979. Differences in the age of Pacific hake (*Merluccius productus*) using whole otoliths and sections of otoliths. J. Fish. Res. Board Can. 36: 141–151.

Dans deux populations de merlus du Pacifique étudiées, les âges des vieux poissons déterminés sur des coupes passant à travers le noyau des otolithes sont supérieurs aux âges déterminés sur la surface de l'otolithe. Toutes les parties de l'otolithe ne croissent pas au même rythme durant la vie du poisson. Le changement dans les modalités de croissance de l'otolithe correspond à peu près à l'âge auquel la croissance en longueur du poisson ralentit. En dépit de la disproportion de croissance en longueur et en hauteur de l'otolithe, ce dernier continue à épaissir avec l'âge, surtout dans sa portion intérieure ventrale. Les âges déterminés sur les coupes semblent plus fiables pour les vieux poissons à cause de ce changement allométrique dans la croissance de l'otolithe en fonction de l'âge des poissons. Nous recommandons d'examiner de façon routinière les coupes d'otolithes dans tous les cas où l'on essaie de déterminer l'âge des poissons au moyen des otolithes.

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A method to determine the age of Pacific hake (*Merluccius productus*) from otoliths has been described by Dark (1975). Annuli were determined by counting translucent zones of whole otoliths viewed in reflected light. He concluded that growth zones could be accurately enumerated, and that ages assigned were reliable. The application of this technique to unexploited Pacific hake stocks in the Strait of Georgia was satisfactory for young hake up to age 3 or 4, but very few fish were found to be older than age 6. However, older fish were observed for the large stocks of Pacific hake found in the Pacific Ocean off the west coasts of Canada and the United States (Dark 1975; Beamish et al. 1976). Thus either the aging method was not applicable to Pacific hake from the Strait of Georgia or these fish suffered a high mortality rate. Aging errors seemed more probable because of problems associated with the identification of annuli on the edge of some otoliths. Otoliths that presented problems appeared thicker than other otoliths in which the annuli were more easily recognized. Many of the thick otoliths contained an area on the edge that varied in width and contained no distinctive opaque or translucent (hyaline) zones.

To verify that the method of counting annuli for Strait of Georgia hake was similar to the method used by Dark (1975) for offshore Pacific hake, samples of Pacific hake were collected from offshore for age determination. Annuli on whole otoliths from these offshore stocks were easier to identify than annuli from otoliths from Pacific hake in the Strait of Georgia. However, despite the facility with which many of these offshore hake could be aged using the method described by Dark (1975), it was difficult to determine the age of some of the larger (and apparently older) Pacific hake. Again, it was noticed that many of the otoliths from the offshore hake that were difficult to age were thicker than otoliths from some of the more easily aged otoliths. Because of this difficulty and the difficulty interpreting the annual growth pattern on the surface of otoliths taken from Pacific hake in the Strait of

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Georgia, this study was undertaken to determine whether sections of otoliths might more accurately represent the true age of these fish.

Materials and Methods

Samples for the comparative aging study were collected with an Engel 434 midwater trawl equipped with a 2-cm stretched mesh codend liner and consisted of the total catch of hake in one haul from the Strait of Georgia and 85% of the hake in a haul from fishing grounds off southwest Vancouver Island. Both samples were selected because they contained a wide size range of hake. All Pacific hake were sexed, fork length was measured, and the largest otolith (sagittae) was removed from each side of the head. The otoliths were cleaned of blood, otic sac, and other membranes and were stored in a vial containing 50% glycerine. Subsequently, approximately 0.5% thymol was added to the 50% glycerine solution to inhibit fungus development.

Whole otoliths were placed in a black dish in the storage solution and viewed under reflected light through a dissecting microscope. Ages were assigned independently for hake from the Strait of Georgia by two experienced readers using one or both otoliths and without additional information. Ages were assigned to offshore hake in the same manner with one additional set of determinations obtained from the aging unit at the National Marine Fisheries Center, Seattle, Wash. Otoliths in which the surface age was uncertain were rejected. No attempt was made to resolve differences in ages between readers, as each age was considered to be the reader's best estimate.

One of a pair of otoliths was removed from the 50% glycerine storage solution and rinsed in toluene prior to embedding in clear epoxy (Hysol brand epoxy patch kit). The epoxy served to prevent fragmenting of thin sections and facilitated handling and mounting of the cut sections. Two dorsal ventral sections (approximately 500 μm thick) were taken from the center of the nucleus (Fig. 1), perpendicular to the anterior posterior axis. Sections were cut using a Bronwill model TSM thin-sectioning machine. This machine employs diamond impregnated, water-cooled grinding disks and will semiautomatically section otoliths at varying thicknesses quickly and efficiently. Cut sections were dried and mounted on glass slides with clear liquid mounting medium (Flo-texx). Mounted sections were examined with a compound microscope using transmitted light. Contrast between opaque and translucent zones was enhanced by using phase contrast or dark field optics. Only one experienced reader was available to determine the age from otolith sections.

Whole otoliths were photographed on Kodak plus X film using a Wild–Leitz dissecting microscope equipped with a 10×13-cm negative camera. Four sources of reflected light were adjusted to provide maximum definition of growth zones. Otolith sections were photographed using transmitted light on Kodak plus X film using a Wild–Leitz phase contrast compound microscope equipped with a 10×13-cm negative camera and/or an Olympus microscope using dark field optics and a 35-mm camera. Better photographs could be obtained if the light transmitted through the section was not completely perpendicular to the section.

The terminology applied to zones that are used to determine age potentially could be confusing in this study because the two methods used require different methods of illumination. The zones identified from either method result from changes in optical density of the otolith. It is generally considered that an opaque and translucent (or hyaline) zone forms approximately annually. Under reflected light the opaque zone appears light and the translucent zone appears dark. When transmitted light is used, as for otolith sections, the opaque zone is dark and the translucent zone is light. In this study the opaque or summer growth zones were counted.

Otolith thickness, length, and height were determined by randomly selecting a subsample of otoliths from each age-class (as determined from sections), or measuring all otoliths in the sample. The otoliths were wiped dry and dial calipers were used to make a series of measurements internal–externally across the nucleus for thickness, dorsal–ventrally across the nucleus for height, and anterior–posteriorally for length (Fig. 1).

Weight was measured after otoliths were dried at 60°C to a constant weight. This took 24 h. Only unbroken otoliths were used for age, weight comparisons.

Results

Strait of Georgia Pacific Hake

Age determination — Whole otoliths were examined by viewing the exterior lateral surface. Zones were visible across the height (dorsal–ventral surface), and the length (anterior–posterior surface). When viewing whole otoliths from the lateral surface, it was impossible to detect all growth zones because in many older fish a distinct growth pattern formed only on the inner surface (Fig. 1).

Ages recorded for Pacific hake from the Strait of Georgia (Table 1) produced a 57% agreement between readers aging whole otoliths, and 93% agreement within ± 1 yr. Agreement between the age determined from whole otoliths and the age determined from the section was 47% identical, and 79% within ± 1 yr. The agreement between readers and between methods was tested using a two-way analysis of variance by treating each age separately. Ages assigned using surface and section ages as well as ages assigned using the surface ages were significantly different ($P < 0.01$).

A comparison of ages of sectioned otoliths with the two surface otolith ages was also made by determining the mean surface otolith age for each section age, and by subtracting the section age from the mean surface age. The mean deviation of the surface age was then plotted against the section age (Fig. 2). Surface otolith ages were similar to section ages up to about age 5. After age 5, the difference between surface otolith ages and section ages increased with increase in section age.

Otolith growth — When the lateral thickness was compared to assigned ages, it was apparent from the slopes of the equations that the thicker otoliths produced older ages by the section method (Table 2). The straight line fitted to the points is significantly linear in all cases (F test, $P < 0.01$). The reduced slope of the equation for section ages indicates that ages assigned

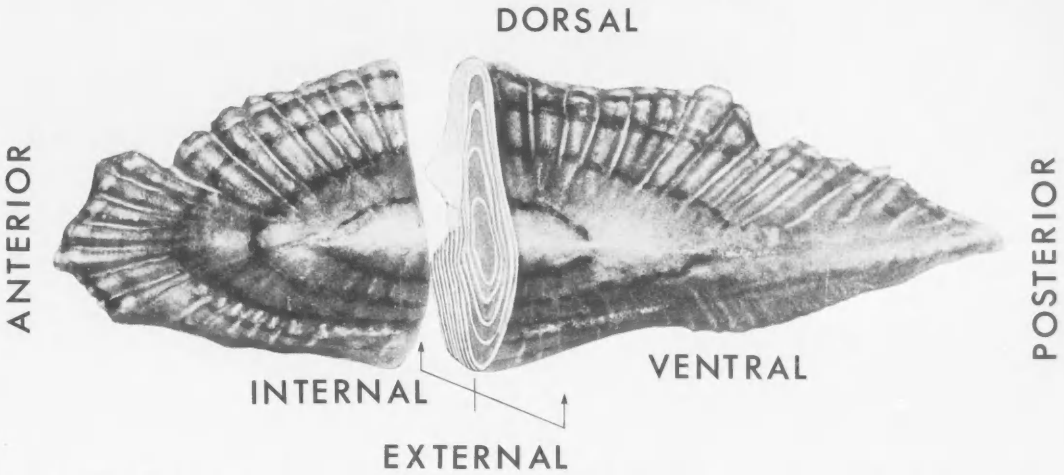

FIG. 1. Typical older Strait of Georgia Pacific hake otolith showing position of section used for age determination. Drawing approximates the relationship of section annuli to surface annuli. Section annuli are most easily counted in the interior ventral quadrant.

using sections have a more gradual increase in thickness with time.

There was a relationship between increase in fish length and increase in otolith size. There was a stong linear relationship between fish length and otolith length ($r = 0.97$), fish length and otolith height ($r = 0.97$), and fish length and otolith thickness ($r = 0.87$). However, otolith growth was not isometric. The ratio of otolith height divided by otolith length decreased with increasing otolith length, indicating the general form was changing from ovate to elongate. For smaller otoliths or younger fish, thickness increased linearly with increase in otolith height (Fig. 3), but for larger otoliths or older fish the thickness continued to increase while the height remained relatively constant (Fig. 3). The variance associated with these measurements was large, but the trend toward an allometric increase in otolith thickness for larger otoliths or older fish is apparent from Fig. 4. A similar, but less obvious relationship was found for otolith thickness and otolith length. It appears that for some otoliths the increase in growth is through increased thickness and not increased height or length. Thus growth associated with larger otoliths or older fish produces zones that are not clearly defined on the periphery of the lateral surface. Because the otolith may continue to increase in thickness, growth zones may be more apparent in cross sections from the vicinity of the nucleus.

Cross sections of otoliths indicate that the increase in thickness results in a deepening of the central groove on the interior lateral surface of the otolith. Growth zones can be distinguished on either side of this central groove, but are most easily distinguished from the interior ventral quadrant (Fig. 1). Phase contrast or dark

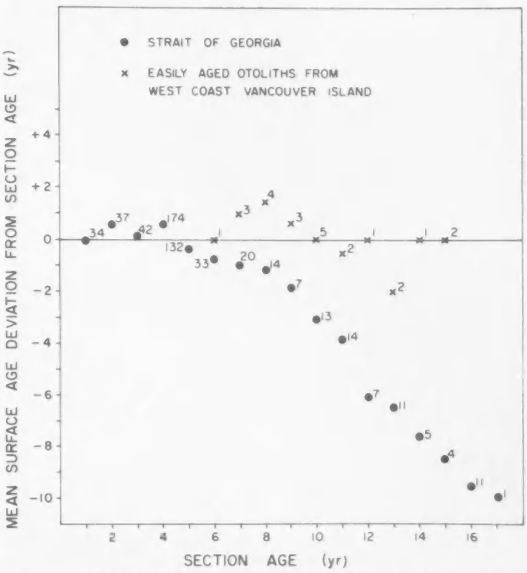

FIG. 2. Deviation of mean age determined using surface otolith readings from ages determined from otoliths sections. For comparison, comparable data determined from very easily aged otoliths from a sample collected off the west coast of Vancouver Island are included. Sample size is indicated for each point.

field microscopy increases the contrast between zones. A pattern of alternating opaque and translucent zones could be identified in most sections. However, the interpretation of these zones from some of the otolith sec-

TABLE 1. Strait of Georgia Pacific hake otolith ages (yr) and corresponding mean length (cm).

	Reader no. 1 – surface									Reader no. 2[a] – surface									Reader no. 2 – section								
	♂			♀			Total			♂			♀			Total			♂			♀			Total		
Age	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n
1							10.0	1.14	40							9.6	0.57	7							10.0	0.14	40
2	24.8	1.13	12	25.2	1.58	10	24.6	0.74	22	26.0	1.26	9	23.5	1.85	4	25.2	1.01	13	27.2	1.15	21	25.7	1.31	17	26.6	0.85	38
3	32.4	1.05	33	29.3	0.96	22	31.2	0.75	55	36.0	2.09	7	30.3	1.32	4	33.9	1.55	11	33.6	1.11	22	32.5	1.07	20	33.1	0.76	42
4	42.4	0.22	91	42.9	0.38	52	42.6	0.19	143	42.6	0.25	49	43.9	0.33	16	42.9	0.21	65	42.2	0.16	101	43.0	0.32	73	42.5	0.16	174
5	43.4	0.20	125	43.9	0.25	79	43.6	0.16	204	44.5	0.71	46	44.4	0.34	25	44.1	0.24	71	43.5	0.26	66	44.1	0.22	57	43.8	0.17	123
6	44.2	0.28	50	44.6	0.33	38	44.4	0.21	88	43.9	0.58	14	45.7	0.33	3	44.2	0.49	17	44.4	0.47	23	45.7	0.71	9	44.8	0.39	32
7	44.4	0.42	17	45.1	0.61	10	44.7	0.34	27	45.5	0.73	8	47.0	2.00	2	45.8	0.64	10	44.7	0.41	11	45.6	0.95	8	45.1	0.45	19
8	44.2	0.64	9	45.7	0.64	7	44.9	0.47	16	45.3	0.85	4	44.5	1.50	2	45.0	0.62	6	45.1	0.44	8	45.0	1.03	6	45.1	0.17	14
9	44.3	0.88	8	43.7	1.67	3	44.1	0.71	11	43.0	—	1	42.0	—	1	42.5	0.35	2	43.8	0.65	6	45.0	—	1	44.0	0.54	7
10	44.4	0.75	5				44.8	0.74	5	46.5	0.50	2				46.5	0.35	2	44.1	0.50	10	46.0	1.22	4	44.6	0.51	14
11																			44.4	0.69	11	45.3	1.33	3	44.6	0.58	14
12																			44.6	0.87	5	47.0	—	1	45.0	0.75	6
13																			45.3	0.68	7	43.5	1.50	2	44.9	0.60	9
14																			44.0	—	1	45.8	0.48	4	45.4	0.46	5
15																			43.3	0.67	3	45.0	2.00	2	44.0	0.75	5
16																			44.9	0.88	7	45.8	1.31	4	45.2	0.68	11
17																			44.0	—	1				44.0	—	1
Total			350			221			611			140			57			204			303			211			554

[a] Reader no. 2 determined the ages from a sample of approximately the first one third of the total sample examined by Reader no. 1.

TABLE 2. Relationship of otolith thickness and surface and section ages for Pacific hake from the Strait of Georgia.

Reader	Method	Sample size	Equation	r
2	section	546	$Y = 0.097 + 0.009X$	0.78
2	surface	203	$Y = 0.079 + 0.145X$	0.74
1	surface	607	$Y = 0.065 + 0.017X$	0.79

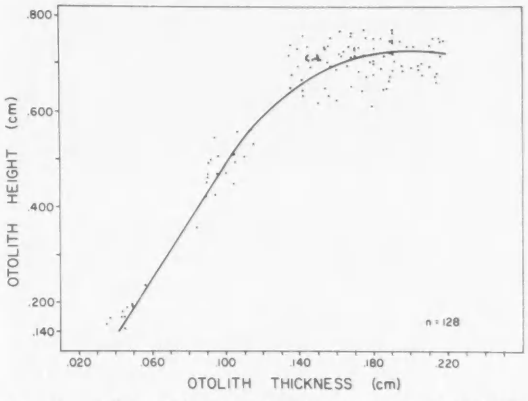

FIG. 3. Relationship of otolith thickness and otolith height for Strait of Georgia Pacific hake from a randomly selected sample of approximately 10 otoliths per age-class, as determined by section ages.

tions was difficult because of the close spacing of zones and the presence of checks or incomplete zones. Occasionally, interpretations were inconsistent when growth zones from different areas of the otolith section were counted. In such cases the ages assigned probably only approximate the actual age of the fish. Many of the sections were moderately easy to age and some of the otolith sections had very prominent growth zones (Fig. 4). Annuli from the surface of older otoliths are less prominent than zones from sections (Fig. 4). Once it was suspected that surface ages were underestimating the actual age, it was possible in some cases to obtain older ages by counting the closely spaced zones toward the anterior or posterior end of the otolith (Fig. 4H). Even if such zones were counted, and they were not visible on all otoliths (Fig. 4F) the surface readings were still lower than the section readings.

OFFSHORE PACIFIC HAKE

Age determination — Otoliths from offshore Pacific hake were more easily aged than otoliths from Pacific hake in the Strait of Georgia. A small percentage of otoliths from offshore Pacific hake had extremely distinct annuli when viewed from the surface. A comparison of the mean deviation of the surface and section ages of these otoliths (Fig. 2 and 5) indicated rather close agreement, with no trend to higher ages using the section method.

Agreement among readers varied. One Canadian reader tended to age fish younger than the other Canadian reader and the United States reader. Because the use of the younger ages might be criticized as an attempt to increase the mean deviation of surface and section ages, and because the United States reader was experienced with the age determination of hake, the determinations of this one Canadian reader have not been included (Table 3). Agreement between the other Canadian reader and the United States reader was 37%, 66% within ±1 yr, and 95% within ±2 yr. A two-way analysis of variance, treating each fish separately and using the readers' ages for each fish, showed that there was no significant difference ($P > 0.05$) between these two determinations. A comparison of otolith section ages and the United States and Canadian surface ages shows 16% agreement, 40% within ±1 yr, 57% within ±2 yr and 20% agreement, 45% within ±1 yr, 57% within ±2 yr, respectively. Both surface readings were significantly different ($P < 0.05$) than the section readings.

The mean deviation of the two surface ages from section ages was similar. After age 11 mean section ages no longer were similar to mean surface ages, and the deviation between the two methods increased with increasing section age, with up to 6 yr difference observed for the oldest fish (Fig. 5). In this particular sample 60% of the fish had a section age older than age 11. If section ages could be considered accurate, then it could be estimated that in this sample there was a possible aging error of up to 6 yr for all fish older than age 11.

In a previous study it was found that for hake off Vancouver Island, the definition of otolith growth zones from individual catches varied among catches within a small area (Beamish 1976). The sample of otoliths used in this study contained an above average percentage of otoliths in which growth zones were difficult to identify from the surface. At this time it was not known what percentage of the population of larger hake found in this area could be considered to be older than age 11, using section ages. It does appear from the preliminary study (Beamish 1976) that the percentage will be important.

Otolith measurements of offshore Pacific hake — The relationship between otolith weight and thickness is probably curvilinear, since the curve must pass through zero. Since only data for older fish are available, a straight line might also be used to describe this portion of the curve where the change in slope is minimal. The straight line regression was significantly linear (F test, $P < 0.01$) with a correlation coefficient of $r = 0.77$ indicating a possible linear relationship between increases in otolith thickness and weight increases for this part of the curve.

No trend was shown when height and length of offshore Pacific hake otoliths were compared to otolith

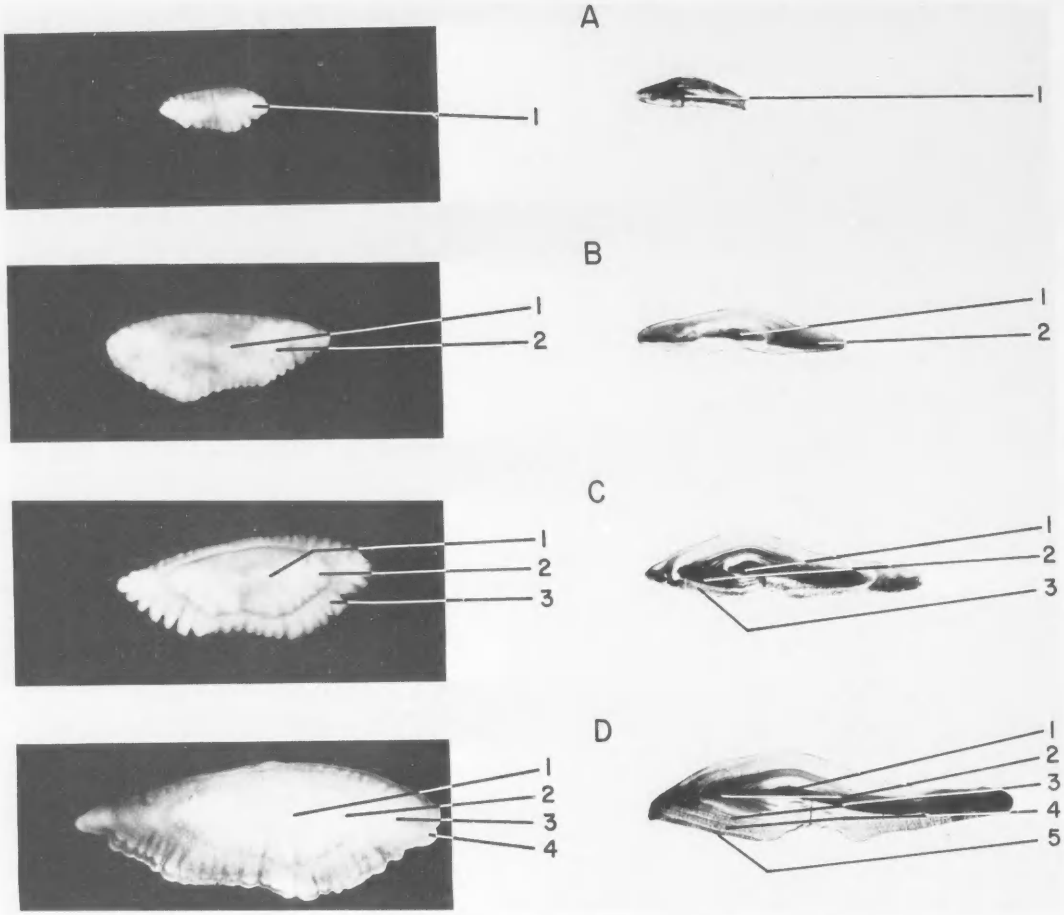

FIG. 4. Whole otoliths from Strait of Georgia Pacific hake showing surface annuli and corresponding section from the second otolith from the same fish. The magnification for whole otoliths was identical and the magnification for the sections was identical, but the magnification for whole otoliths and otolith sections are not equal. Otoliths A through H were selected from very easily aged otolith sections so that a range of section ages would be represented. In both methods opaque zones were counted as annuli. Other interpretations may appear possible especially with ages assigned using the surface annuli. However, ages indicated represent the original interpretation of the reader and higher surface ages were not considered until section ages were known. The definition of growth zones in the photographs is not as clear as that observed using a microscope.

thickness (Fig. 6A, only length shown). In a comparison of otolith length and height with otolith dry weight (Fig. 6B, only length shown) some increase in length with increasing otolith weight was apparent. There was a more well-defined relationship (F test, $P < 0.01$, Fig. 7) between otolith weight and thickness.

It appears that growth in length or height is either greatly reduced or that the annual increments are proportionally smaller than corresponding increments in thickness. In contrast, the close relationship between thickness and weight indicates that growth of older otoliths continues, but that increases in thickness become proportionally more important than increases in length or height.

Increases in section age were not closely related to increases in otolith length (Fig. 8A) or height. Increases in section age showed a slightly stronger relationship with increases in thickness (Fig. 8B). The relationship comparing surface ages to otolith length and height was similar to the section age–thickness relationship.

The relationship between otolith weight and surface ages determined by the United States reader was poorly

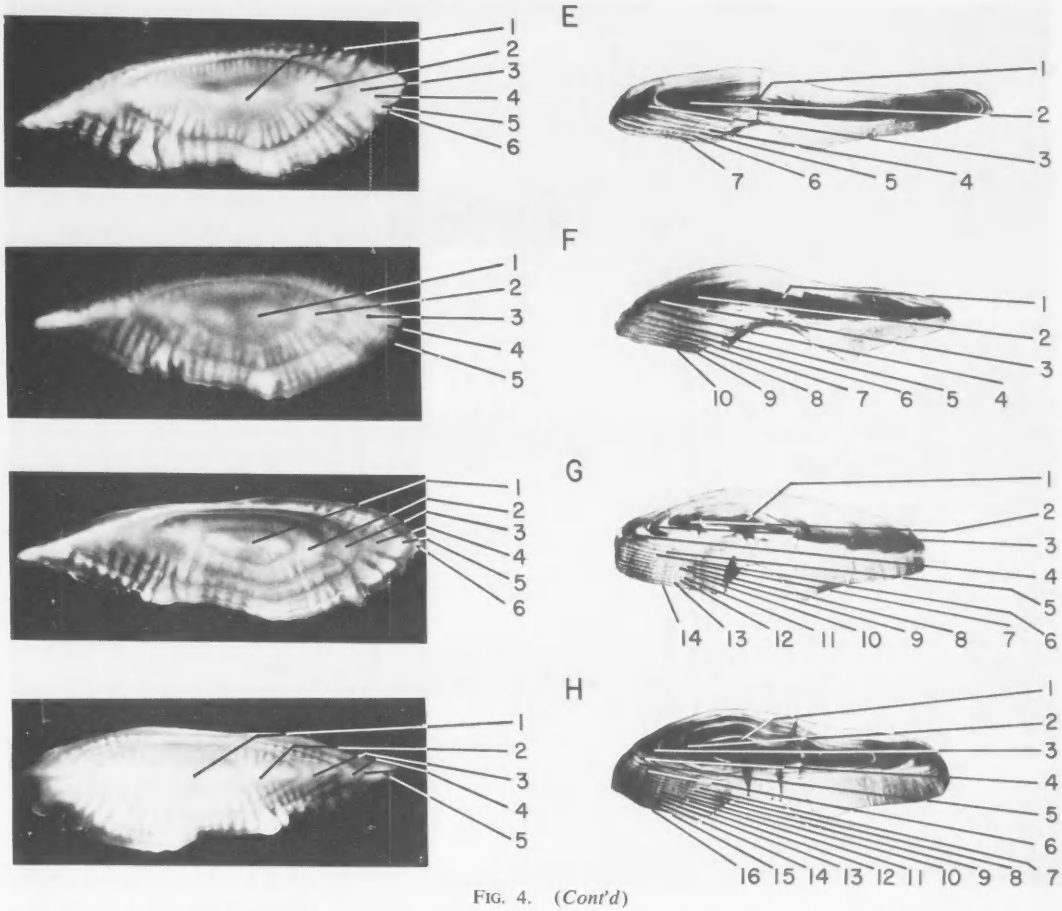

FIG. 4. (*Cont'd*)

defined but significant (F test, $P < 0.01$; $r = 0.66$). The Canadian surface age determinations were more weakly correlated to weight than the United States readings but were also significantly linear, (F test, $P < 0.01$; $r = 0.58$). Section ages were (F test, $P < 0.01$; $r = 0.51$) related to weight but the correlation coefficient was low.

It was apparent that otoliths from older offshore Pacific hake increased in size through increases in thickness more than by increases in length or height, and that at least some of the older fish had thicker and heavier otoliths. However, the relationship of otolith thickness to section age was not as obvious for offshore Pacific hake as for Pacific hake from the Strait of Georgia. Pacific hake in the Strait of Georgia were smaller and the otoliths were shorter (the range of otolith length for mature Strait of Georgia fish was 1.8–2.3 cm compared to 1.8–2.8 cm for offshore fish). Because fish from both areas reached similar ages, the better agreement between surface and section ages found for offshore hake appears to result from their longer period of growth in otolith length.

Discussion

The data presented in this study cannot prove conclusively that the ages assigned are accurate, but there is little doubt that age determinations using whole otoliths will not accurately determine the age of most older Pacific hake in some stocks, and some older Pacific hake in other stocks. The interpretation of a yearly pattern from otolith sections can be difficult, but it appears to present a more accurate account of age than results for whole otoliths, particularly when thick otoliths with poorly defined annuli on the otolith margin are encountered.

If only the ages from the surfaces of otoliths from the offshore populations that were easily read were compared with ages from sections of these otoliths, it was found that assigned ages were in close agreement.

TABLE 3. Offshore Pacific hake otolith ages (yr) and corresponding mean length (cm).

	Canadian Reader – surface									United States Reader – surface									Canadian Reader – section								
	♂			♀			Total			♂			♀			Total			♂			♀			Total		
Age	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n
5													57.0	—	1	57.0	—	1				54.0	—	1	54.0	—	1
6	56.0	—	1	53.8	0.25	4	53.6	0.25	5	51.0	—	1	57.0	3.00	3	53.0	0.71	4				54.4	0.93	5	54.4	0.93	5
7	54.0	1.16	3	54.3	0.99	7	54.2	0.74	10	51.7	0.67	3	56.2	1.13	9	55.1	1.03	12	55.5	1.50	2	54.5	1.31	6	54.8	1.01	8
8	52.0	0.41	4	55.1	0.81	20	54.6	0.72	24				55.5	0.83	13	55.5	0.83	13	55.5	2.50	2	55.0	0.96	11	55.1	0.86	13
9	54.1	1.12	7	56.1	0.63	28	55.7	0.62	35	55.5	2.72	4	56.5	0.61	24	56.4	0.63	28	53.5	0.50	2	54.6	0.85	11	54.5	0.72	13
10	52.7	0.80	6	57.2	0.86	22	56.3	0.78	28	55.0	3.00	2	56.9	1.28	16	56.7	1.17	18	53.0	1.53	3	56.3	1.00	10	55.5	0.91	13
11	58.0	5.00	2	57.7	0.97	23	57.7	0.94	25	55.7	1.33	3	58.6	1.03	20	58.2	0.93	23	52.0	—	1	57.1	1.18	7	56.5	1.21	8
12	55.5	1.50	2	57.3	1.21	9	57.5	0.84	11	54.0	—	1	58.1	1.08	12	57.8	1.04	13	57.0	6.00	2	58.0	1.20	11	57.9	1.22	13
13	57.0	—	1	60.4	0.96	8	60.0	0.93	9				58.6	1.16	8	58.6	1.64	8	53.6	1.08	5	59.1	0.92	21	58.1	0.88	26
14				61.0	2.78	6	61.0	2.78	6										55.0	3.00	2	56.7	0.81	28	56.6	0.77	30
15				62.0	—	1	62.0	—	1										54.0	1.47	4	58.7	1.22	18	57.9	1.09	22
16																			54.0	—	1	58.0	1.70	5	57.3	1.54	6
Total			26			128			154			14			106			120			24			134			158

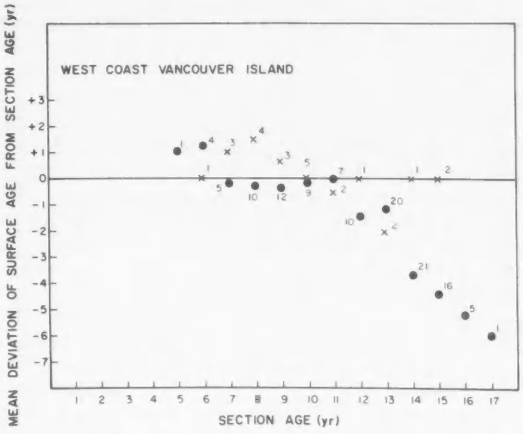

FIG. 5. Deviation of mean age determined using surface otolith readings and ages determined from otolith sections for offshore hake. For comparison, comparable data determined from very easily aged otoliths from the same sample are included in this figure and Fig. 2. Sample size is indicated for each point.

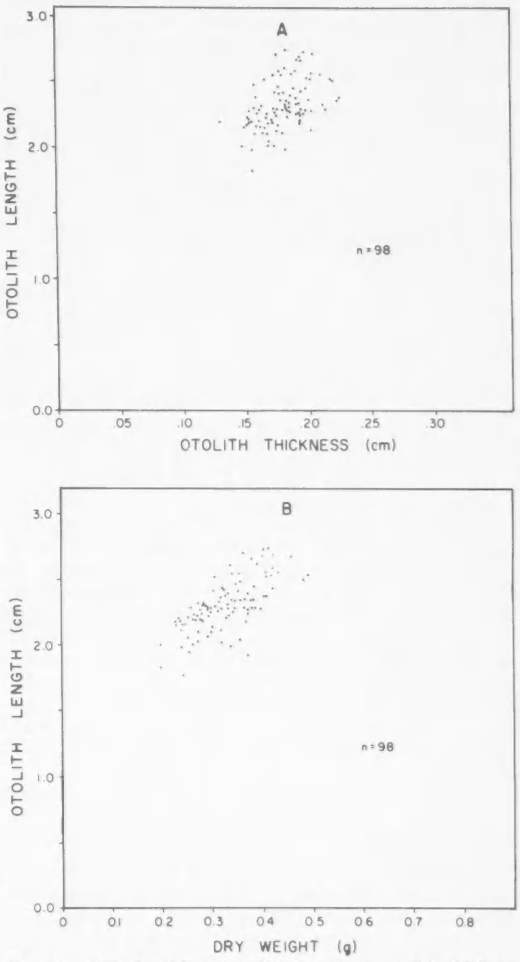

FIG. 6. Relationship of otolith length to otolith thickness and dry weight for Pacific hake sampled off the west coast of Vancouver Island.

It was primarily the otoliths that were difficult to age from the surface that produced ages lower than the otolith section ages. There also was good agreement between section ages and surface ages of immature fish. Dark (1975) validated the ages obtained from surface readings for offshore Pacific hake for young and "middle aged" fish. He observed a strong 1961 year-class that persisted as an identifiable year-class until 1969, and was able to confirm that ages were accurate at least up to age 8. He also found by examining the margin of the otoliths from young fish that only one opaque zone and one hyaline zone formed each year. In this study, there was good agreement between otolith section ages and surface ages for young fish from the same population studied by Dark. If the annuli identified by Dark (1975) can be considered to be validated up to age 8, and the ages from sections agree with surface ages for younger fish, then it should follow that the zones from sections also form annuli — at least for the younger fish.

The section age at which surface and section ages no longer were similar was lower for Pacific hake from the Strait of Georgia. Mature hake in the Strait of Georgia average about 44 cm long (Beamish et al. 1976), while offshore hake average about 54 cm long (Beamish 1976). Lengths of immature hake in the Strait of Georgia averaged approximately 12 cm age 1, 24 cm age 2, 36 cm age 3 (Beamish 1976), and offshore Pacific hake averaged 15 cm age 1, 28 cm age 2, and approximately 37 cm age 3 (Dark 1975). Thus the size of fish from these two stocks was similar up to the age at which they first mature. Offshore hake, however, continue to grow longer, and reach larger sizes. Agreement between surface and section ages also continued longer for offshore hake. It appears that the growth zones observed in otolith sections form annually and continue to form annually after the increase in fish length becomes reduced. However growth zones formed on the otolith surface become difficult to identify as annuli once major increases in fish length are reduced.

Underestimating the age of hake in the Strait of Georgia has important biological and management implications. It is possible, also, that the ages assigned to Pacific hake found to the south of the Strait of Georgia population in Puget Sound (Kimura and Millikan 1977) may also underestimate the actual ages of older fish. Section ages indicate that Pacific hake stocks in the

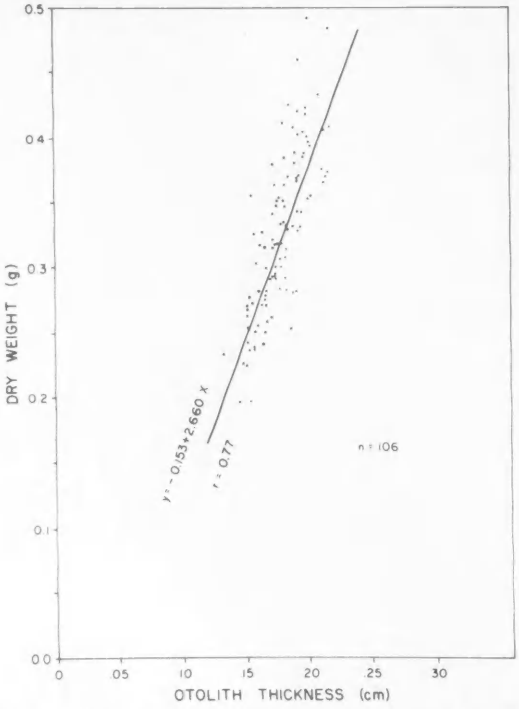

FIG. 7. Relationship of otolith dry weight to otolith thickness, surface age and section age for Pacific hake sampled off the west coast of Vancouver Island. Surface ages determined by the Seattle age determination unit were used.

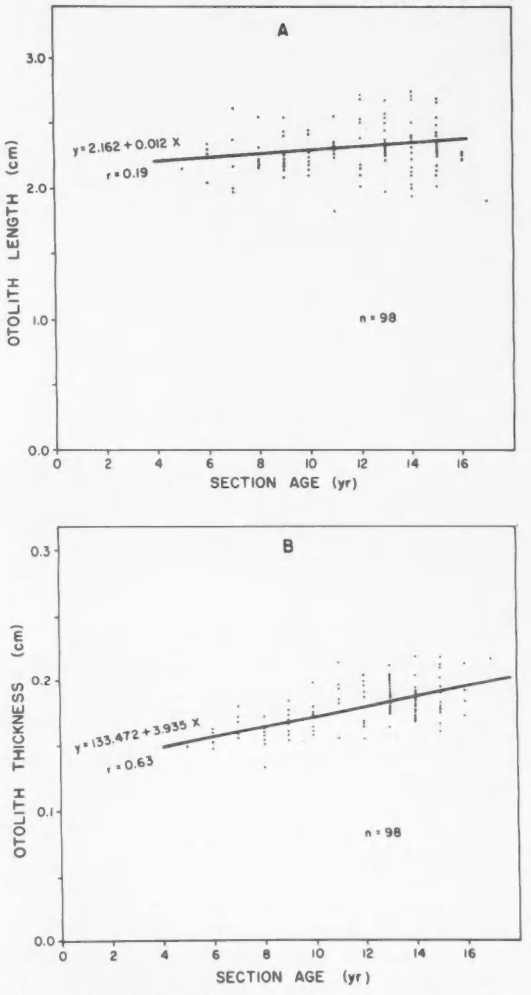

FIG. 8. Relationship of otolith length and thickness to section age for Pacific hake sampled off the west coast of Vancouver Island.

Strait of Georgia are composed of older fish and will have lower rates of annual natural mortality than reported earlier (Beamish 1976). However, the pattern of growth will be similar to that already described, since the older fish increase very little in length after sexual maturity.

It is questionable whether the aging errors indicated in this study will have an important effect on the management of the bulk of the offshore hake population, unless it could be shown that the behavior, relative biomass, and distribution of the larger and older fish had some special biological or economic importance. Biologically, it is interesting to know that hake can reach ages older than age 13 found by Dark (1975) from samples off Washington State. It is also interesting that a population that has been fished extensively still contains larger and older fish. However, Pacific hake off the west coast of Vancouver Island are thought to be only a small part of one large offshore population (Utter and Hodgins 1971), and the bulk of this population are believed to be smaller and younger than those found in the more northern waters (Alverson and Larkins 1969; Dark 1975).

There is the more general consideration about the implications of this and other similar studies to accepted age determination procedures. A relationship between otolith growth and fish growth was observed in this study and in other studies for other species (e.g. Trout 1954; Harden Jones 1968), and for hake by Hickling (1933), and Dark (1975). In this study otolith size was shown to be related to fish size, but all areas of an otolith did not increase proportionately as the otolith increased in size. Otoliths from older fish showed reduced growth in length and height, but continued to increase in thickness and weight. The increase in thickness occurs primarily through deposition on the internal surface, and growth zones are most readily distinguished

ventrally. This increase in thickness of an otolith has been observed for flatfishes, and *Sebastes* spp. (Blacker 1974), for horse mackerel (*Trachurus trachurus*) (Blacker 1974), giant bluefin tuna (*Thunnus thynnus thynnus*) (Butler et al. 1977), and several freshwater species (Power 1978; Beamish unpublished data). The results of these studies and the present study indicate that an examination of the surface of an otolith may not provide as reliable ages as previously thought. Therefore, it would seem wise to examine at least some sections of otoliths when any serious attempts are being made to describe the age composition of a stock. Otoliths that are larger and thicker than average and have indistinct annuli on the margins should most certainly be sectioned. A reassessment should also be made of the validity of extrapolating "aging validation" procedures for younger fish to older fish. If the method is good only for young fish, it should be made clear that there is an upper age limit above which the method has not been validated.

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Estimating Chlorophyll Extraction Biases

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A simplified chlorophyll analytical procedure is described that requires 4 min/sample and features an overall precision (measured by the coefficient of variation, C.V.) of ~3% at the 15 mg/m^3 chlorophyll *a* level. The C.V. varies inversely with concentration for spectrophotometric determinations. The molar extinction coefficients (665 nm) for chlorophyll *a* were estimated to be 2.5–6.0% greater in acetone (90%) compared to methanol (100%) and 2.5% greater in a 1:1 mixture (v/v) of methanol-dimethyl formamide compared to methanol. Acetone (90%) extractions of naturally occurring mixtures of the common bloom-forming blue-green algae *Microcystis*, *Oscillatoria*, *Aphanizomenon*, and *Anabaena* yielded erratic recoveries of chlorophyll *a*. The lowest recovery (34%) occurred with an *Oscillatoria* population. On the one occasion when a four-way solvent extraction comparison was performed, acetone (90%) failed to extract 29 ± 2% of the chlorophyll *a* in a mixture of *Anabaena*, *Microcystis*, and *Aphanizomenon*, whereas the other three solvent preparations yielded identical (complete) extractions. Methanol or acetone-dimethyl sulfoxide are recommended for chlorophyll extraction from blue-green algae, while acetone-dimethyl sulfoxide is recommended for diatoms or Chlorophyta populations.

Key words: chlorophyll, Cyanophyta, Chlorophyta, chlorophyll analysis, methanol, acetone, methanol-dimethyl formamide, acetone-dimethyl sulfoxide

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Nous décrivons le protocole analytique simplifié pour la chlorophylle, qui ne prend que 4 min par échantillon et est caractérisé par une précision globale (mesurée par le coefficient de variation, C.V.) de ~3% à une concentration de chlorophylle *a* de 15 mg/m^3. Le C.V. varie inversement avec la concentration dans les déterminations spectrophotométriques. On estime que les coefficients d'extinction molaire (665 nm) pour la chlorophylle *a* sont de 2,5 à 6,0% plus élevés dans l'acétone (90%) que dans le méthanol (100%) et 2,5% plus élevés dans un mélange méthanol-diméthyl formamide de 1:1 (v/v), que dans le méthanol. Les extractions à l'acétone (90%) de mélanges naturels des Cyanophycées à fleur d'eau communes *Microcystis*, *Oscillatoria*, *Aphanizomenon* et *Anabaena* donnent des récupérations erratiques de chlorophylle *a*. La récupération la plus faible (34%) s'est produite avec une population d'*Oscillatoria*. En une occasion, alors qu'on effectuait une comparaison d'extraction par solvants a quatre voies, l'acétone (90%) n'a pas réussi à extraire 29 ± 2% de la chlorophylle *a* dans un mélange d'*Anabaena*, *Mycrocystis* et *Aphanizomenon*, alors que les préparations aux trois autres solvants donnèrent des extractions identiques (complètes). Nous recommandons le méthanol ou l'acétone-diméthyl sulfoxyde pour l'extraction de la chlorophylle des Cyanophycées, alors que pour les populations de diatomées ou de Chlorophyta, nous recommandons l'acétone-diméthyl sulfoxyde.

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ALTHOUGH evidence has been accumulating that acetone (90%) does not reliably and quantitatively extract the chlorophyll *a* in limnetic phytoplankton populations, limnologists have generally ignored the potential extraction biases resulting from the application of standardized acetone procedures developed for oceanography (Strickland and Parsons 1968). Acetone (90%) has been widely employed partly because of convention, and partly because of the superior spectral data for chlorophyll pigments in that solvent (Vernon 1960). Shoaf and Lium (1976) showed that a 1:1 mixture of dimethyl sulfoxide (DMSO) and acetone (90%) yielded more complete extractions of green algae as compared to the standard reference solvent (90% acetone), but reported equal extraction results for selected laboratory blue-green algae cultures. Methanol (MeOH) has also been suggested as an extractant (Strickland 1965; Talling and Driver 1961; Tett et al.

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1975); both Marker (1972) and Talling (1969) have reported superior extractions of planktonic algae with MeOH as compared to acetone. Volk and Bishop (1968) reported the special suitability of N,N-dimethyl formamide (DMF) for extracting the chlorophyll in the eucaryotic benthic thermal alga *Cyanidium caldarium*. Marker (1977, 1972) has discussed some of the special spectrophotometric characteristics of chlorophyll *a* and pheophytin *a* in MeOH.

The purpose of this investigation was to estimate the extinction coefficients at 665 nm for chlorophyll *a* in methanol, methanol-DMF, acetone, and acetone-DMSO and to compare the ability of these solvents to extract chlorophyll *a* from natural phytoplankton populations.

Materials and Methods

Sample Collection

Epilimnion water samples for chlorophyll analysis were collected from eutrophic Lakes Mendota and Delavan in southern Wisconsin during fall 1971, summer–fall 1972, fall 1973, and July 1977. Replicate 1-L polyethylene sampling bottles were filled on station at a fixed depth (usually 1 m) using a submersible pump.

Routine Chlorophyll *a* Extraction Procedure

The following procedure is recommended for routine chlorophyll *a* determinations for eutrophic lakes. This procedure was used for analysis of the samples collected prior to 1977. The 1-L samples were filtered through a 5.5-cm 934AH Reeve Angel glass fiber filter (Long and Cooke 1971) using an aspirator vacuum. The filter pad was inserted into a 2.5 × 10.5-cm glass vial and 40 m of extracting solvent added. The capped vial was allowed to stand overnight at 4°C in the dark, then shaken and returned to the dark cold room to stand upright for an additional 24 h without disturbance. After warming to room temperature, the extract was decanted directly into a 5-cm cuvette for spectrophotometric analysis. The transfer step must be accomplished smoothly and in one motion to avoid raising the settled floc from the bottom of the vial.

Evaluation of Extinction Coefficients

Let the following four solvent preparations be denoted A, M, A-DMSO, and M-DMF, respectively: 1) acetone (90%):water (10%); 2) MeOH (100%); 3) a 1:1 mixture by volume of acetone (90%) and DMSO; 4) a 1:1 mixture by volume of MeOH and DMF. To compare the extinction coefficients of equimolar concentrations of chlorophyll *a* in acetone vs. MeOH, two 8-L water samples (collected July 1977) were filtered using Reeve Angel glass fiber filters, and the chlorophyll was extracted with 35 mL of either MeOH (100%) or acetone (100%). The water retained on the filter pads resulted in a final percent composition of solvent water of 80:20. After centrifugation, the highly concentrated clear chlorophyll *a* parent extracts were used in parallel dilution experiments to estimate the ratio of the molar extinction coefficient of chlorophyll *a* in acetone (90%) as compared to MeOH (100%). Five-mL and 10-mL aliquots of each parent were diluted to 100 mL in volumetric flasks, using both the same and the contrasting solvent type. The dilution scheme (Table 1) allows comparison of equimolarity chlorophyll extracts in acetone vs. MeOH.

Table 1. Final solvent compositions[a] of diluted chlorophyll parent extracts.

Final % of parent	Parent A		Parent M	
	Acetone (90%)[b]	MeOH (100%)[b]	Acetone (90%)[b]	MeOH (100%)[b]
5%	89.5:0:10.5	4:95:1	85.5:4:10.5	0:99:1
10%	89:0:11	8:90:2	81:8:11	0:98:2

[a]Acetone:MeOH:H_2O in percent.
[b]Solvents used for dilutions.

The ratios of the extinction coefficients of chlorophyll *a* in M-DMF as compared to M, and A-DMSO as compared to A were estimated by comparing the absorbances (665 nm) of M and A parent extracts diluted 50% by pure DMF and DMSO, respectively, with the absorbances of the undiluted parents. The dilutions employed volumetric flasks to ensure final molarities exactly one half of the parent values.

Comparison of Solvent Extraction Efficiencies for Chlorophyll *a*

Twelve replicate 1-L water samples (collected July 1977) were filtered individually through 4.25-cm Reeve Angel 934AH glass fiber filters (two pads required) and extracted with 20 mL of one of the four solvent preparations, A, M, A-DMSO, or M-DMF. After extracting overnight in the dark at 4°C, the extracts were centrifuged, and the clear supernatants were quantified spectrophotometrically at 665 nm and 750 nm using a Beckman DU spectrophotometer adjusted to a slit width of 0.3 and equipped with 5-cm cuvettes. The 750-nm measurement was used as an extract turbidity correction (Strickland and Parsons 1968). Additional discrete spectral measurements were made on selected extracts in the spectral range of 660–670 nm.

Statistical Analysis

Spectrophotometric response model — The statistical testing of differences in solvent extraction completeness is facilitated by examining a response model. Equation 1 describes the instrumental response (665-nm absorbance, 5-cm light path, corrected for the 750-nm turbidity blank) associated with the filtered-extracted natural water sample. A total of N sample replicates (litre bottles) are partitioned among the I solvent types.

$$X_{ij} = c_i p_i(\mu + e'_{ij}) + \epsilon_{ij} \quad (1)$$

where:

X_{ij} is the spectrophotometric response (absorbance) for the jth replicate of the ith solvent type;

μ is the true mean concentration of chlorophyll *a* at the time and place of sampling;

c_i is a solvent-dependent coefficient relating absorbance to chlorophyll *a* concentration in the original water sample.

p_i is the fraction of chlorophyll *a* extracted by the ith solvent.

e'_{ij} is the sampling error for the ijth sample;
ϵ_{ij} is the analytical error.

The coefficient c_i depends on the molar extinction coefficient of chlorophyll *a* in the solvent (Beer's law), the water volume extracted, the solvent extraction volume, and the light path-length. Because all factors except the solvent-dependent extinction coefficients remain constant, $R_{ij} = c_i/c_j$ estimates the ratio of the molar extinction coefficients for solvents i and j.

Expanding equation 1 and expressing the sampling error in terms of final absorbance (e_{ij}), results in equation 2. The sampling and analytical errors are independent and additive; thus, the

$$X_{ij} = c_i p_i \mu + e_{ij} + \epsilon_{ij} \tag{2}$$

"sample variance" $S^2_{\cdot\cdot}$ estimates the sum of the analytical and sampling errors.[3] Furthermore, because the factors c_i and p_i appear in a multiplicative fashion, neither the p_i's nor the contrasts $(p_i\text{-}p_j,\ i \neq j)$ can be estimated without independent knowledge of the c_i's. Based on the monochromatic chlorophyll *a* equation in Strickland and Parsons (1968, p. 194), acetone (90%) extracts of pure chlorophyll *a* yield the following equation for the extraction procedure described: $\text{Chl}_a\ (\text{mg/m}^3) = 83.0\ (X_{663}\text{-}X_{750})$.

Equations 1 and 2 are valid only in the absence of pigment interferences for chlorophyll *a*. This condition is never strictly true for pigment extracts of natural phytoplankton populations. However, in some cases interferences can be neglected. The monochromatic equation (Strickland and Parsons 1968, p. 194) is especially well suited to chlorophyll extracts of the Cyanophyta since this algal class does not contain chlorophyll *b* or *c*, and the important phycobilin pigments do not interfere with chlorophyll *a* determinations. The phycobilin pigments interfere with determinations of chlorophylls *b* and *c* using the trichromatic equations; hence, these equations cannot be used for mixed chlorophylls *a* and *b*, and/or *c*, and a slight underestimation populations. Use of the monochromatic equation leads to a slight overestimation of chlorophyll *a* in a mixture of chlorophylls *a* and *b*, and/or *c*, and a slight underestimation of the sum of the three chlorophylls.

Significant interference by pheophytin *a* is detectable following Strickland and Parsons (1968). However, the smaller acidification ratio (90% acetone) of pure chlorophyll *b* (Loftus and Carpenter 1971) implies an overestimation of pheophytin *a* and an underestimation of chlorophyll *a* using spectrophotometry when chlorophyll *b* is initially present.

Estimating differences in chlorophyll extraction completeness — A statistical test for significant differences in the p values can be constructed based on departures of the observed absorbance ratios from unity. The test is developed for A and M; analogous results hold for other solvent comparisons.

Let the spectrophotometric responses for A and M extracted samples be denoted by *x*'s and *y*'s, respectively. For each date, these statistics are independent observations from independent X and Y distributions, where Var X = Var Y. The statistic U, defined by equation 3, is used for testing statistical hypotheses concerning the ratio (p_1/p_2). The parameter R_1 is the absorbance ratio for equimolarity chlorophyll solutions

$$\text{U} = \{|(\bar{X}/\bar{Y})| - R_1|\} * 100 \tag{3}$$

in A and M. By the independence of $\bar{X}/\bar{Y}$ and R_1, Var U is given by equation 4 (Hogg and Craig 1970).

$$\text{Var U} = 10^4 \text{Var}\,(\bar{X}/\bar{Y}) + \text{Var}\,R_1 \tag{4}$$

In the present problem, the variance of $\bar{X}/\bar{Y}$ can be estimated approximately using equation 5 and the definition of the C.V.[4]

$$\text{C.V.}\,(\bar{X}/\bar{Y}) \simeq \frac{\sqrt{2}}{\sqrt{n}}\,\text{C.V.}\,(X) \tag{5}$$

In equation 5, n is the number of sample replicates used in estimating each mean in the ratio. The uncertainty in R_1 is estimated independently using parallel dilution experiments.

The null hypothesis defined by $p_1 = p_2$ (A and M are equally effective in extracting chlorophyll) is accepted or rejected for a specified level (type I error), depending on the experimental value of U and $\hat{\sigma}_u$, the estimated standard error for U. The approximate 95% confidence intervals defined by $(0 + 2\,\hat{\sigma}_u)$ depend on n and $\bar{x}$, since the C.V. for $\bar{x}$ decreases with increasing $\bar{x}$.

Results

Peak extract absorbances were observed at 665 nm for M, M-DMF, and A-DMSO, and at 663-664 nm for A. Addition of 1 drop concentrated HCl to the A and A-DMSO extracts caused a 2-nm spectral shift in peak absorbance to higher wavelengths.

The 665-nm acidification ratios for epilimnion acetone (90%) extracts generally ranged from 1.70 to 1.75, indicating no significant amount of pheophytin *a* in the epilimnion algal populations extracted. On June 10, 1972 ratios of 1.61 to 1.65 were observed; these reduced ratios are attributed in part to large amounts of chlorophyll *b* in species of Chlorophyta codominant (with *Anabaena*) on that late spring date. On July 22, 1977 89% of the acetone-extracted pigment was chlorophyll *a*, the residual 11% being pheophytin *a*.

The pooled variance in X was estimated $(s^2_{\cdot\cdot})$ to be $24 * 10^{-6}$ for $(0 \leq X \leq 0.6)$, based on 31 degrees freedom (df) and 16 different sampling dates. For absorbances above 1.00, $s^2_{\cdot\cdot}$ increased to $\sim 100 * 10^{-6}$. A principal source of analytical error involved the 750-nm corrections; these were usually in the range 0.005–0.010 and showed consistency on individual dates. The 750-nm corrections were thus determined only on a subset of the sample extracts. The C.V. (X) was ~3% at the 15 mg/m^3 chlorophyll *a* level and decreased with

[3]Upper case letters are used to denote random variables and lower case letters to denote actual observations following standard statistical notation.

[4]Equation 6 is based on the variance of the product of two random variables (Goodman 1960), and the near equivalence of the C.V. for X and $1/Y$ for the application of interest. The C.V. for X is defined as $100\ s_x/\bar{x}$ where s_x is the observed sample standard deviation and $\bar{x}$ is the observed mean.

TABLE 2. Parallel dilution experiment—absorbance ratios[a] for equimolarity chlorophyll *a* solutions in acetone vs. methanol.

	Responses[a]			
	Parent[b] M		Parent[b] A	
Diluting[b] solvent	10%	5%	10%	5%
Acetone	0.3720	0.1840	0.3580	0.1800
MeOH	0.3625	0.1780	0.3405	0.1715
Absorbance ratios[c]	1.026 ± .014	1.034 ± .028	1.051 ± .014	1.050 ± .028

[a]At 665 nm, corrected for blank.
[b]The percentages are the molar percent *of the parent* in each of the final dilutions. Diluting solvents are acetone (90%) or MeOH (100%).
[c]The indicated uncertainties in the ratios are twice the estimated standard error for the ratio estimates (see text).

increasing chlorophyll concentration to a lower bound of about 1%.

Evaluation of Extinction Coefficients

Comparison of absorbance ratios after parallel dilution of acetone and methanol parent extracts of chlorophyll *a* was used to detect possible differences in the extinction coefficient of chlorophyll *a* in acetone vs. methanol. The best estimate of the 665-nm ratio of the extinction coefficients, $R_1 = (A/M)$, is between 1.012 and 1.065 (Table 2). Differences between the estimated ratios for the two investigated dilution levels (5% vs. 10%) are not statistically significant. However, ratios based on the acetone parent are significantly greater than for the MeOH parent. The minor percentage of pheophytin *a* has a negligible bias effect on the estimated chlorophyll *a* extinction ratio for the two solvents.

After correcting the parents' absorbances for the slight (~1.5%) negative departures from response linearity (vs. concentration) at the .950 absorbance level, the extinction coefficient of chlorophyll *a* in A-DMSO is seen to be indistinguishable from the value in A (Table 3). However, chlorophyll *a* has a slightly higher 665-nm extinction coefficient in M-DMF as compared to M (+2.5%). Thus, the ratio $R_2 = 1.025$ (chlorophyll *a* in M-DMF vs. M). The present work confirms Shoaf and Lium's assertion that the extinction coefficients are identical in A and A-DMSO.

TABLE 3. Absorbance effects of diluting methanol parent extract with DMF and acetone parent extract with DMSO.

Absorbances and ratios	
M[a] = .951/2	A[a] = .910/2
M-DMF[b] = .495	A-DMSO[b] = .464
R[c] = 1.041 ± .014	R[c] = 1.018 ± .014

[a]Parents.
[b]Chlorophyll molarity is exactly ½ of parent.
[c]See note c of Table 2.

Evaluation of Solvent Extraction Efficiencies

The statistic $U_1 = 100(\bar{X}^*_2/\bar{Y}^*_1 - R_1)$ is an unbiased estimator for the percent difference in extraction completeness of A-DMSO vs. M. In fact, the two experimental values, $u_1 = +.2$ or -2.2, are obtained depending on whether we adopt 1.051 or 1.026 as the best estimate for R_1 (Table 2). Given the statistical uncertainties in the estimated ratios, it is apparent that A-DMSO and M resulted in statistically equivalent extractions of the blue-green algae population.

Similarly, the statistic, $U_2 = 100(\bar{Y}^*_2/\bar{Y}^*_1 - R_2)$ estimates the percent difference between the extraction of M-DMF vs. M. The experimental value $u_2 = -.3$, a value not significantly different from zero. The inference is that M and M-DMF, hence M, M-DMF, and A-DMSO, all gave equivalent extractions of the mixed *Microcystis-Aphanizomenon-Anabaena* population on July 22, 1977.

The multiple solvent extraction comparison on samples collected July 1977 resulted in strikingly larger extract absorbances for A-DMSO, M and M-DMF replicates as compared to the A replicates (Table 4). After correcting the mean responses for the more important negative departures from Beer's Law at absorbance level = 1.4, it is apparent that A-DMSO gave a superior extraction (as compared to A) by 40 ± 2%. The ratio $\bar{X}^*_2/\bar{X}^*_1$ (see Table 4 notes) is an unbiased estimator for the extraction ratio because of the common spectral characteristics of chlorophyll in A vs. A-DMSO.

Systematic extraction comparisons between acetone (90%) and MeOH were also performed on 13 earlier dates on samples collected prior to 1977. On a majority of the dates (8) the two solvents yielded indistinguishable results (Table 5). On two dates MeOH gave superior yields; on three dates acetone gave higher results. However, on only two dates were the extraction results markedly different. Acetone failed to extract 65% of the chlorophyll in a September *Oscillatoria* bloom in Lake Delavan. Conversely, in late October

TABLE 4. Comparison of solvent extraction of blue-green algae in Lake Mendota.

	Solvent extractant[a]			
	A	A-DMSO	M	M-DMF
Replicates	1.083	1.457	1.408	1.448
	1.066	1.456	1.433	1.451
	1.079	1.448	1.402	1.440
Means[a]	$\bar{x}_1 = 1.076$	$\bar{x}_2 = 1.454$	$\bar{y}_1 = 1.414$	$\bar{y}_2 = 1.446$
Standard[b] errors	$s_{x_1} = 0.009$	$s_{x_2} = 0.005$	$s_{y_1} = 0.016$	$s_{y_2} = 0.006$
Ratios[c]	na	$\bar{x}_2^*/\bar{x}_1^* = 1.398 \pm .019$	$\bar{y}_1^*/\bar{x}_1^* = 1.359 \pm .019$	$\bar{y}_2^*/\bar{y}_1^* = 1.023 \pm .014$
	na	$\bar{x}_2^*/\bar{y}_1^* = 1.028 \pm .014$		

[a] X_1, X_2, Y_1, Y_2 are random variables assigned to the absorbance response of the four solvent extractants. The starred variables (for example, $\bar{x}_1^*$) have been corrected for the slight negative departures from response linearity (as a function of chlorophyll concentration) for absorbances exceeding ~0.800.

[b] The sample variances $s^2 = s_t^2/2 + s_r^2$ where s_t^2 is the pure spectrophotometric instrumental error, and s_r^2 is the residual lake sampling and analytical (extraction procedure) error. s_t^2 was estimated as $0.36 * 10^{-4}$ at the absorbance = 1.4 level based on 12 df.

[c] The indicated uncertainties in the ratios are equivalent to *twice* the estimated standard error for the ratio statistic.

TABLE 5. Solvent extraction contrasts (A vs. M) for 13 sampling dates prior to 1977.

Date	Lake	n	$\bar{x}$	$\bar{x}/\bar{y}$	u	Conf. Int.[a]	Signif.[b]	Phytoplankton extracted[c]
1971								
Sept. 29	Mendota	5	0.180	1.013	−2.7	±3.7	—	*Microcystis*, filamentous diatom, *Aphanizomenon*, *Anabaena*
1972								
May 4	Mendota	3	0.120	1.092	−5.2	±6.8	—	centric diatom bloom
May 9	Mendota	4	0.200	1.036	−0.4	±3.8	—	centric diatom bloom
June 10	Mendota	3	0.385	1.008	−3.2	±2.5	*	*Anabaena*, Chlorophyta species
July 31	Mendota	5	0.375	1.042	+0.2	±2.2	—	*Microcystis*, *Aphanizomenon*
Sept. 21	Mendota	2	0.063	1.086	+4.6	±15.7	—	*Microcystis*, *Aphanizomenon*, *Lynqbia*, *Anabaena*, *Staurastrum*
Sept. 27	Mendota	2	0.110	1.037	−0.3	±8.9	—	same as on Sept. 21, 1972
Oct. 7	Mendota	3	0.185	1.089	+4.9	±5.0	—	not determined
Oct. 13	Mendota	3	0.155	1.165	±12.5	±6.2	**	*Microcystis*, centric diatom, *Staurastrum*, *Aphanizomenon*
Oct. 25	Mendota	2	0.175	1.048	+0.8	±6.0	—	same as on Oct. 13, 1972
Sept. 23	Delavan	4	0.260	0.380	−66.0	±3.0	*	*Oscillatoria* bloom
Oct. 26	Delavan	3	0.360	2.040	+100.0	±4.7	**	cetric diatom bloom
1973								
Sept. 24	Mendota	2	0.320	1.197	±15.7	±4.8	**	*Microcystis*, *Aphanizomenon*

[a] The approximate 95% confidence interval for u is given by $(u + 2\hat{\sigma}_u)$. See text.

[b] * indicates that MeOH gave a more complete extraction (vs. acetone); ** indicates a more complete extraction using acetone (90%); — indicates no significant difference between the two solvents.

[c] Genera are listed in the approximate order of their abundance, based on microscopic examination of slides. A "bloom" denotes a nearly unalgal population. When only two genera are listed they can be considered codominant.

1972, acetone yielded much improved results over MeOH for a centric diatom bloom. The systematic extraction comparisons for the Delavan *Oscillatoria* bloom were the result of qualitative observations of incomplete acetone extractions of the same *Oscillatoria* population on September 4. Similar qualitative observations of incomplete acetone extractions were also made on an *Aphanizomenon* bloom in Lake Mendota during fall 1970. The physiological conditions responsible for incomplete acetone extractions are apparently both species-dependent and time-dependent for individual species.

Discussion

The expeditious chlorophyll extraction procedure described in this paper has a C.V. (~1–3%) which is profitably compared with a C.V. = 22% reported by Platt and Filion (1973). The present procedures avoid solvent transfer steps, employ glass fiber filters, and allow extraction of a large integrative sample. A small

amount of a liquid organic base like diethyl amine (1 mL/L solvent) can be added as a safeguard against extract acidity. The avoidance of $MgCO_3$ and the use of glass fiber filters and tall extract vials allow elimination of the usual centrifugation step.

The literature on chlorophyll extinction coefficients in MeOH is contradictory. Tett et al. (1975, p 893) asserted that the specific extinction coefficient (chlorophyll *a*) is about 15% less in MeOH than in 90% acetone. On the previous page the same authors calculated an MeOH extinction coefficient based on diethyl ether, which when compared with Vernon's (1960) widely accepted extinction coefficient for acetone (90%), implied a (acetone/MeOH) ratio of 1.265. Marker (1972) discussed literature estimates of the 665-nm ratio ranging from 1.09 to 1.17. The best evidence from the parallel dilution experiments reported here indicates a lower ratio (1.03 to 1.06).

Moss (1967) noted that changing an acetone:H_2O mixture from 9:1 to 8:2 (volume basis) should not affect the extinction coefficient of chlorophyll *a* by more than 3%. Similarly, Vernon (1960) noted the insignificant effect of 5% diethyl ether on the chlorophyll extinction coefficient in acetone. Because of the similar dielectric constants for acetone and MeOH (21.4, 33.1, Daniels and Alberty 1963, p. 502), minor percentages of either MeOH or acetone in the contrasting solvent should have had only minor effects on our estimated ratios of chlorophyll extinction coefficients (Tables 1–3).

From Tables 4 and 5 it is clear that if the true (A/M) extinction coefficient ratio was as large as 1.17, then we could logically infer that MeOH had yielded a significantly improved chlorophyll extraction (vs. acetone) on all but two of the dates studied. The larger ratio would also imply (speciously) that A-DMSO had yielded incomplete chlorophyll extractions.

Methanol or A-DMSO are recommended for routine chlorophyll extractions of blue-green algae. Both solvents rapidly and quantitatively extract all pigments from the planktonic blue-green algae tested, yielding a white floc. The chlorophyll extraction completeness of acetone (90%) is not directly verifiable because some of the masking phycocyanin pigment remains in the floc. An important advantage of A-DMSO over MeOH is that the 665-nm acidification ratio for A-DMSO remains suitable for pheophytin *a* corrections (Marker 1977; Shoaf and Lium 1976). However, for large-scale work the volatile MeOH is easier to work with, and the acidification ratios need be estimated on only a small subset of the epilimnion chlorophyll samples. It appears that A-DMSO is best suited to estimating chlorophyll in diatoms and particularly the Chlorophyta (Shoaf and Lium 1976). Using the statistical methods in this paper and following Cochran (1963), ratio estimation can be used to eliminate gross chlorophyll extraction biases in cases where one solvent yields a complete extraction and another yields low results.

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Tissue Sources and Diagnostic Value of Circulating Enzymes in Cetaceans

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GERACI, J. R., AND D. J. ST. AUBIN. 1979. Tissue sources and diagnostic value of circulating enzymes in cetaceans. J. Fish. Res. Board Can. 36: 158–163.

Tissue activities of alanine aminotransferase (AlAT), aspartate aminotransferase (AspAT), sorbitol dehydrogenase (SDH), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), and creatine kinase (CK) were determined in three cetacean species: the bottlenosed dolphin, *Tursiops truncatus*, beluga whale, *Delphinapterus leucas*, and harbor porpoise, *Phocoena phocoena*. Liver was the principal source of AlAT and SDH activity; GGT and ALP were concentrated in kidney. Muscle tissues showed the highest CK and AspAT activity, although most other tissues had appreciable AspAT activity. Based on tissue specificity, plasma AlAT and SDH are recommended for use in diagnosing hepatic disorders. Plasma GGT may have potential value in assessing the type of hepatobiliary damage caused by trematodes in cetaceans; ALP has no obvious advantage over GGT. Creatine kinase elevations in plasma would indicate muscle damage or exercise stress, while plasma AspAT may reflect either muscle or liver release and must be used in conjunction with other more tissue-specific enzymes. Reference plasma enzyme activities determined in healthy captive *T. truncatus* showed significant procedure-related differences that must be considered when circulating enzymes are used in research or diagnosis.

Key words: enzymes, tissues, plasma, Cetacea, stress

GERACI, J. R., AND D. J. ST. AUBIN. 1979. Tissue sources and diagnostic value of circulating enzymes in cetaceans. J. Fish. Res. Board Can. 36: 158–163.

Nous avons déterminé chez trois espèces de cétacés, le dauphin à gros nez, *Tursiops truncatus*, le béluga, *Delphinapterus leucas*, et le marsouin, *Phocoena phocoena*, l'activité des enzymes suivantes dans les tissus : alanine aminotransférase (AlAT), aspartate aminotransférase (AspAT), sorbitol déshydrogénase (SDH), gamma glutamyl transférase (GGT), alcaline phosphatase (ALP) et créatine kinase (CK). Le foie est la source principale d'activité de AlAT et de SDH; GGT et ALP sont concentrées dans le rein. Les tissus musculaires montrent la plus grande activité de CK et de AspAT, bien que dans la plupart des autres tissus il y ait activité appréciable de AspAT. Sur la base de la spécificité tissulaire, on recommande l'AlAT et la SDH plasmatiques pour la diagnose des désordres hépatiques. La GGT plasmatique peut avoir une valeur dans l'évaluation du type de dommage hépatobiliaire causé par les trématodes dans les cétacés; la AlAT ne semble pas l'emporter sur la GGT. Des élévations de créatine kinase dans le plasma seraient un signe de dommage musculaire ou de stress résultant d'un travail, alors que la AspAT plasmatique peut refléter la mise en liberté dans le muscle ou le foie et doit être utilisée conjointement avec d'autres enzymes plus spécifiques quant au tissu. Des activités de référence d'enzymes plasmatiques déterminées chez des *T. truncatus* captifs en bonne santé montrent des différences significatives liées à la méthode d'analyse et qui doivent être prises en considération quand des enzymes en circulation sont utilisées dans la recherche ou la diagnose.

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ENZYMES are normally present in plasma as a result of cell turnover and leakage into the circulation. Tissue destruction, whether physiological or pathological in nature, often leads to an increase in circulating enzyme levels (Wilkinson 1962; Batsakis and Briere 1967). Once the source of the enzyme is known, it can be used to localize the site and determine the extent of organ damage.

Plasma enzymes have been used as clinical tests for organ damage in many mammalian species (Kaneko and Cornelius 1970) for which the tissue distributions are known and in others, including cetaceans, for which data are completely lacking and interpretations are tenuous at best. This study was undertaken to determine the tissue distribution of six enzymes in three cetacean species: the bottlenosed dolphin, *Tursiops truncatus*, beluga whale, *Delphinapterus leucas*, and the harbor porpoise, *Phocoena phocoena*. Reference plasma activities necessary for the interpretation of clinical results were determined in healthy captive *T. truncatus*. The enzyme creatine kinase (CK) (EC 2.7.3.2) was ex-

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amined because of its proven value in assessing physiological muscle damage of the type that might result from exertion and stress (Geraci and Medway 1973). The other enzymes, aspartate and alanine aminotransferases (AspAT and AlAT) (EC 2.6.1.1 and EC 2.6.1.2), sorbitol dehydrogenase (SDH) (EC 1.1.1.14), gamma glutamyl transferase (GGT) (EC 2.3.2.2), and alkaline phosphatase (ALP) (EC 3.1.3.1) signal liver damage in man and other mammals. Liver is a common site of parasitic infection in cetaceans (Delyamure 1955) and would also be one of the target organs for damage by toxicants and pollutants found in the marine environment (Gaskin et al. 1973).

Materials and Methods

Two adult (IM,IF) *D. leucas* were killed by Inuit hunters near Tuktoyaktuk, N.W.T., in July 1975. All principal tissues (detailed in Tables 1–6) were removed within 90–120 min of death and frozen immediately in liquid N_2. Samples from four (two adult; two subadult) harbor porpoises, *P. phocoena*, were collected from animals shot in the Bay of Fundy near New Brunswick in August 1976. Tissues were frozen in liquid N_2 within 10–20 min of death. The frozen samples were transported to the laboratory in liquid N_2 and then transferred to a conventional freezer for storage at −25°C for 4 mo (*P. phocoena*) to 7 mo (*D. leucas*) prior to analysis. Related studies on the storage of seal tissues for enzyme analysis indicate no significant change in the distribution patterns under these conditions (Austin 1976; St. Aubin and Geraci 1977).

Tissues from two *T. truncatus* were obtained from research and exhibit institutions in California. One dolphin drowned accidentally, and samples were collected and frozen within 3 h of death. The other died shortly after a capture-related stress episode; tissues for enzyme assay were collected and frozen within 20 min of death. All samples were stored at −25°C for 4 mo prior to analysis. The problem inherent in using the two captive animals for such studies is recognized, but *T. truncatus* is a valuable and protected species for which no other source of tissues is available.

Thirteen apparently healthy adult and subadult *T. truncatus* held at the New England Aquarium (NEA), Boston, Mass., the Montreal Aquarium, Montreal, Que., and Marineland of the Pacific, Palos Verdes, Calif., were blood-sampled for plasma enzyme activity between March and June 1976. Plasma was removed from the heparinized blood after centrifugation, generally within 1 h of collection, frozen and transported to Guelph for analysis. The samples were stored at −25°C for up to 1 mo prior to assay.

During the period 1973–78, 36 blood samples were drawn from the NEA *T. truncatus* for processing by commercial clinical laboratories, using multichannel autoanalyzers. All other enzyme assays were performed manually using commercially available reagent kits, according to the prescribed methodology.

Aminotransferase activity was measured using BMC (Boehringer Mannheim GmbH, Mannheim, Germany) test kits 15955 and 15956 for AspAT and AlAT, respectively. BMC test kits 15721, 15987, and 15522 were used for CK, ALP, and GGT, respectively. Activity is reported in milli-international units (mU), defined as the conversion of one millimicromole of substrate per minute. Sorbitol dehydrogenase was assayed using Sigma test kit 50-UV (Sigma Chemical Co., St. Louis, Mo.); results are expressed in Sigma units (SU), representing the number of millimicromoles of NADH oxidized per hour at 25°C under the manufacturer's specified reaction conditions. All determinations were performed on a Coleman 55 spectrophotometer with temperature control (Coleman Instrument Co., Maywood, Il).

Tissue homogenates were prepared using the method of Herzfeld and Greengard (1971). Structural components were removed to reduce the amount of nonparenchymatous tissue in the sample. Mucosal surfaces of the stomach and duodenum were dissected away from the submucosa and serosa. Cartilaginous elements of the lung and conspicuous ducts in the liver and pancreas were removed.

Samples of tissue weighing 1.5–2.0 g were placed immediately into 5 mL of cold phosphate-buffered saline (PBS, pH 7.5–7.6, 9.5 mM PO_4^{3-}). The tissues were chopped coarsely, then homogenized with a Polytron blender (Brinkmann Instruments, Rexdale, Ont.) at about 15 000 rpm for 15–20 s at 22°C. Homogenates were brought up to volumes ranging from 30 to 40 mL with PBS. Triton X-100 (J. T. Baker Chemical Co., Phillipsburg, N.J.) was then added to a final concentration of 0.5% v/v, and the samples were held for 30–40 min at 4°C. They were then sonicated with an ultrasonic generator (Blackstone Ultrasonics Inc., Sheffield, Penn.) at maximum power for 30 s. The fibrous elements were removed by centrifugation at $1110 \times g$ for 10 min at 22°C. The supernatants were decanted and stored at 4°C, and analyzed for enzyme activity using the same techniques described for plasma.

Results

The results of the tissue distribution studies are grouped according to the organs having the highest activity of each enzyme. We found these to be liver, muscle, and kidney.

Alanine aminotransferase activity in all three species was highest in the liver, although there was marked interspecies variation in absolute values (Table 1). *Phocoena phocoena* liver activity was 3–10 times greater

TABLE 1. Tissue distribution of alanine aminotransferase (in mU/mg fresh weight) in three cetacean species.

Tissue	*P. phocoena* (n = 4)	*D. leucas* (n = 2)	*T. truncatus* (n = 1)
Liver	91 (72–104)[a]	10;20	54
Heart	42[b]	<1;4	3
Kidney	22 (13–24)	2;2	6
Muscle	18 (3–37)	2;2	3
Adrenal	26 (12–34)[c]	1;1	—
Epidermis	3 (2–5)	<1;<1	<1
Brain	—[d]	<1;<1	—
Pancreas	—	<1;<1	<1
Others[e]	dermis blubber	lung stomach intestine thyroid	lung erythrocytes

[a]Average and range. [b]n = 1. [c]n = 3. [d]— = not analyzed. [e]Other tissues having <1 mU/mg.

TABLE 2. Tissue distribution of sorbitol dehydrogenase (in Sigma units/mg fresh weight) in three cetacean species.

Tissue	*P. phocoena* (n = 4)	*D. leucas* (n = 2)	*T. truncatus* (n = 2)
Liver	1175 (852–1639)[a]	137;296	307;1696
Kidney	342 (178–624)	34;342	38;234
Thyroid	—[b]	121;110	—
Epidermis	48 (27–79)	<20;<20	69;143
Muscle	36 (8–59)	<20;<20	<20;<20
Heart	26[c]	20;41	26;124
Adrenal	22 (3–58) [d]	<20;48	—
Stomach	—	<20;44	—
Lung	—	23;<20	<20;<20
Pancreas	—	<20;23	—
Brain	—	<20;<20	—
Others[e]	dermis blubber	intestine	erythrocytes

[a]Average and range. [b]— = not analyzed. [c]n = 1. [d]n = 3. [e]Other tissues having <20 SU/mg. The figure was taken as the lowest limit of significance relative to liver activity.

TABLE 4. Tissue distribution of aspartate aminotransferase (in mU/mg fresh weight) in three cetacean species.

Tissue	*P. phocoena* (n = 4)	*D. leucas* (n = 2)	*T. truncatus* (n = 2)
Liver	144 (103–189)[a]	51;71	79;114
Heart	150[b]	21;59	88;138
Muscle	98 (82–123)	10;21	33;47
Adrenal	52 (48–55)[c]	6;15	—
Pancreas	—[d]	13;24	13;43
Kidney	61 (47–68)	6;12	26;35
Epidermis	20 (11–35)	6;<4	18;10
Brain	—	12;19	—
Stomach	—	<4;12	—
Thyroid	—	7;6	—
Intestine	—	<4;7	—
Spleen	—	—	—
Others[e]	dermis blubber	lung	lung erythrocytes

[a]Average and range. [b]n = 1. [c]n = 3. [d]— = not analyzed. [e]Tissues having <4 mU/mg.

than that of *D. leucas*; the single *T. truncatus* sample was intermediate. Heart, kidney, skeletal muscle, and adrenal showed moderate AlAT activity, particularly in *P. phocoena* in which these tissues were actually more active than *D. leucas* liver.

Sorbitol dehydrogenase was most concentrated in liver in *P. phocoena* and *T. truncatus*, with relatively high activity in kidney (Table 2). Liver activity was lower in *D. leucas* than in the other two species, and in one of the belugas the SDH activity in the liver was exceeded by that in the kidney. Other active tissues included heart and epidermis in *T. truncatus* and thyroid in *D. leucas*.

Creatine kinase activity was most concentrated in the skeletal muscle of all species examined (Table 3). High activity was also found in heart muscle. Moderate activity was present in most tissues assayed, but only as a small percentage of that found in muscle.

Most tissues contained appreciable AspAT activity; liver, heart, and muscle generally exhibited the highest concentrations (Table 4). Tissues from *D. leucas* had less AspAT activity than those from *P. phocoena* and *T. truncatus*.

Gamma glutamyl transferase activity was concentrated primarily in kidney in all three species examined (Table 5). Activity in most other tissues was very low; only liver and adrenals in *P. phocoena* contained appreciable activity relative to kidney.

Alkaline phosphatase was analyzed only in *P. phocoena* (Table 6). Kidney and adrenals showed the highest activities. Of the other tissues examined, only liver had assayable activity, approximately 10% of that of kidney and adrenal glands.

TABLE 3. Tissue distribution of creatine kinase (in mU/mg fresh weight) in three cetacean species.

Tissue	*P. phocoena* (n = 4)	*D. leucas* (n = 2)	*T. truncatus* (n = 2)
Muscle	4627(2803–8562)[a]	3227[b]	2031;905
Heart	1190[b]	571;713	941;533
Brain	—[c]	110;121	—
Stomach	—	70;69	—
Epidermis	51 (41–65)[d]	178;26	62[b]
Kidney	37 (41–56)	<10;<10	25;14
Liver	35 (14–56)	<10;<10	<10;<10
Pancreas	—	17;23	<10;<10
Adrenal	18,28	<10;<10	—
Intestine	—	13;16	—
Others[e]	blubber dermis	thyroid lung	erythorcytes lung

[a]Average and range. [b]n = 1. [c]— = not analyzed. [d]n = 3. [e]Other tissues having <10 mU/mg.

TABLE 5. Tissue distribution of gamma glutamyl transferase (in mU/mg fresh weight) in three species of cetaceans.

Tissue	*P. phocoena* (n = 4)	*D. leucas* (n = 2)	*T. truncatus* (n = 2)
Kidney	49 (35–60)[a]	22;10	18;15
Liver	8 (6–11)	2;<1	5;2
Adrenal	16 (2–35)[b]	<1;<1	—
Epidermis	2(<1–3)	<1;<1	<1;<1
Stomach	—[c]	1[e]	—
Lung	—	<1;<1	1;<1
Intestine	—	<1;<1	—
Others[d]	muscle heart[e] dermis blubber	muscle heart pancreas brain thyroid	muscle heart pancreas erythrocytes

[a]Average and range. [b]n = 3. [c]— = not analyzed. [d]Other tissues <1 mU/mg. [e]n = 1.

TABLE 6. Tissue distribution of alkaline phosphatase (in mU/mg fresh weight) in harbor purpoises, *P. phocoena*.

Tissue	*n*	ALP activity
Kidney	4	42 (29–58)[a]
Adrenal	3	38 (3–58)
Liver	4	4 (3–5)
Heart	1	1
Others[b]	3	epidermis
	3	dermis
	4	blubber
	4	muscle

[a]Average and range. [b]Other tissues having <1 mU/mg.

Plasma enzyme activities in clinically healthy captive *T. truncatus* are shown in Table 7. Although expressed in the same international units of activity, enzyme values reflect procedural differences when results from different laboratories are compared. Only plasma SDH, which was assayed using the Sigma procedure in both our laboratory and the commercial clinical laboratory, showed no significant difference. All other comparisons were significant at the $P < .001$ level.

TABLE 7. Plasma enzyme activities in captive, healthy *T. truncatus*. Result obtained using manual techniques described in this study are compared with values from the same animals assayed by multichannel autoanalyzers.

	Manual techniques (13 dolphins)		Autoanalyzer (8 dolphins)	
Enzyme	*n*	$\bar{x} \pm$ SD (range)	*n*	$\bar{x} \pm$ SD (range)
AlAT (mU/mL)	13	11 ± 4 (7–19)	30	38 ± 19 (10–103)
AspAT (mU/mL)	13	74 ± 43 (33–190)	36	278 ± 112 (100–621)
GGT (mU/mL)	13	16 ± 6 (7–31)	—	—
ALP (mU/mL)	13	127 ± 75 (34–280)	29	445 ± 291 (101–1155)
CK (mU/mL)	12	54 ± 21 (24–94)	—	—
SDH (SU/mL)	13	210 ± 120 (29–435)	8	204 ± 129 (0–336)

Discussion

There is no absolute basis for comparison of the results presented in this study with other species examined, including other marine mammals in our laboratory using identical analytical procedures (Austin 1976; St. Aubin and Geraci 1977). It was not considered necessary to establish optimum enzyme conditions (i.e. pH, temperature, substrate concentrations, etc.) for each species. From a practical point of view, the results are functional in that they reflect a pattern of tissue distribution that is not likely to alter with analytical procedure, and they were done in accordance with techniques adopted in human and veterinary clinical laboratories, both for plasma and tissue distribution studies (Wroblewski and LaDue 1956).

Liver diseases feature prominently in wild and captive cetaceans (Woodard et al. 1969; Medway et al. 1966; Greenwood and Taylor 1977). Enzymes conventionally used to assess liver damage in man and domestic animal species (Wilkinson 1962; Kaneko and Cornelius 1970) have been liberally applied to whales and dolphins. Medway et al. (1966) found persistently and markedly elevated serum AlAT and AspAT associated with jaundice in a *T. truncatus*. This study confirms their presumption of the hepatic origin of those enzymes. As in most other mammalian species, A1AT is the more liver-specific of the two, and increased circulating levels are virtually diagnostic of hepatocellular damage. Liver AlAT activity is inversely related to body size of a given species (Cornelius 1963); this was apparent in our small sample.

Aspartate aminotransferase is more ubiquitously distributed, but is nonetheless helpful in diagnosing liver damage when used in conjunction with other, more liver-specific enzymes (Medway et al. 1966). Ridgway et al. (1970) found plasma elevation of AspAT associated with liver trematodes in *Delphinus* sp.; AlAT and ornithine carbamyl transferase were concurrently elevated. Ornithine carbamyl transferase is highly concentrated in the liver of seals (St. Aubin and Geraci 1977) and domestic species (Ford 1965), and one of us (D. St. Aubin unpublished results) has found the same distribution in a *T. truncatus*. Plasma elevation of AspAT without a concurrent rise in other liver-specific enzymes can also reflect damage to muscle, the other principal source of the enzyme. This is well established in man (Chinsky et al. 1956) and domestic animals (Kaneko and Cornelius 1970), and Ridgway et al. (1970) recorded plasma AspAT elevations in a case of myositis in a porpoise, later identified as *T. truncatus* (S. H. Ridgway personal communication).

Sorbitol dehydrogenase, for which there is no cetacean literature, is as liver-specific in cetaceans as it is other species (Freedland et al. 1965; Zinkl et al. 1971), including seals (St. Aubin and Geraci 1977). Kidney and thyroid, other potential sources of the enzyme, are not likely to contribute to plasma activity. The SDH released by the kidney would likely be excreted (Kemp and Laursen 1962), and any thyroid contribution would be insignificant.

Gamma glutamyl transferase and ALP, though concentrated primarily in kidney, are more useful in the diagnosis of liver disease, with particular reference to the biliary tree (Kaneko and Cornelius 1970, Ford 1974). Medway et al. (1966), however, found no elevation in serum ALP in clinical jaundice, presumably of hepatic origin, in a *T. truncatus*. We cannot assess the clinical value of ALP, as it is influenced by a variety of conditions (Kaneko and Cornelius 1970). Gamma glutamyl transferase may be more useful than ALP because of its more restricted distribution in liver as the potential source of plasma elevations. Elevations in kidney disease are rare (Rosalki 1975), and in contrast with other species (Ford 1974) including seals (Austin 1976), pancreas does not seem to be a principal source of the enzyme in the cetaceans examined. Gamma glutamyl transferase bears investigation particularly

TABLE 8. Comparison of enzyme activities in cetacean and pinniped integuments based on identical assay techniques for each enzyme.

	Cetaceans (epidermis)			Pinnipeds (epidermis + dermis)[a]		
	P. phocoena ($n = 4$)	*D. leucas* ($n = 2$)	*T. truncatus* ($n = 2$)	*Halichoerus grypus* ($n = 6$)	*Phoca hispida* ($n = 6$)	*Phoca groenlandica* ($n = 4$)
CK	51 (45–67)[b]	26;178	62[c]	—[d]	4 (2–4)	1 (1–2)
SDH	48 (27–79)	7;13	69:143	14 (3–23)[e]	3 (0–14)	11 (0–16)
AspAT	20 (11–35)	4;6	18;19	1 (1–2)	1 (1–2)	2 (0–3)
AlAT	3 (2–5)	1	1	1	1	1
GGT	2 (1–3)	1	1	—	1	—

[a]Pinniped data from T. P. Austin and D. J. St. Aubin, M.Sc. theses, University of Guelph, 1976. [b]Average and range. [c]$n = 1$. [d]Not analyzed. [e]$n = 5$.

with respect to biliary disease commonly associated with hepatic trematodes in cetaceans (Woodard et al. 1969).

Creatine kinase was examined for its potential in assessing muscle damage, including that resulting from rigorous exercise and stress (Griffiths 1966). Its specificity for muscle is unequivocal in cetaceans as in other species, and increased circulating levels can reflect muscle damage. Geraci and Medway (1973) found moderate elevations of CK associated with induced exercise in a *T. truncatus*. Serial determinations of this enzyme are recommended to differentiate pathological from physiological elevations (Hudgson et al. 1967).

An interesting and unexpected feature of this study was the enzymatically active nature of cetacean epidermis. This tissue is not normally assayed in enzyme distribution studies, but was included here because of its unique morphology and dynamic adaptation to seasonal needs in cetaceans (Ling 1974). Our only basis for comparison is with results obtained on seal skin (epidermis and dermis) using identical assay procedures. Table 8 shows the overwhelming difference in enzyme activities between cetaceans and pinnipeds, particularly for CK, SDH, and AspAT. These enzymes are, respectively, involved with high energy phosphate conversion, hexose metabolism, and transamination of amino acids. This high enzyme activity is the first biochemical evidence supporting the concept of a metabolically active cetacean epidermis (Ling 1974), whose incompletely understood functions likely surpass that of the keratinized phocid epidermis, which represents a simple mechanical barrier against the water environment.

The disparity in results of plasma enzyme analyses between manual and automated techniques demonstrates the compelling need to establish reference values in accordance with individual analytical techniques, especially when circulating enzymes are used as research tools. Other published "normal ranges" show similar procedure-related discrepancies (Medway et al. 1966; Ridgway et al. 1970) and are indicative of the general lack of standardization in analytical technique (Wilkinson et al. 1972). For clinical purposes, elevations associated with tissue destruction often exceed the variation due to assay method, minimizing but not eliminating the need to establish reference values for each laboratory.

Dynamic studies on enzyme release are required in relation to known and quantifiable organ damage. Such data are not available. Hence, we must rely on the tissue specificity of each enzyme assuming that, as in other species, there will be a correlation between concentration, damage, and release. In this context, AlAT and SDH are liver-specific and increased circulating levels would signal acute hepatic damage. This has already been shown with AlAT (Ridgway et al. 1970). Aspartate aminotransferase is widely distributed and alone is of relatively little value. The hepatic source of GGT may be diagnostically useful in assessing the type of hepatic damage that commonly results from parasitic trematodes in cetaceans. Alkaline phosphatase has no obvious advantage over GGT in the diagnosis of such conditions. Finally, CK has a ubiquitous distribution in muscle, and may be useful in evaluating exercise stress associated with handling free-ranging and captive cetaceans (Geraci and Medway 1973).

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Lethal and Sublethal Effects of Binary Mixtures of Cyanide and Hexavalent Chromium, Zinc, or Ammonia to the Fathead Minnow (*Pimephales promelas*) and Rainbow Trout (*Salmo gairdneri*)[1]

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BRODERIUS, S. J., AND L. L. SMITH, JR. 1979. Lethal and sublethal effects of binary mixtures of cyanide and hexavalent chromium, zinc, or ammonia to the fathead minnow (*Pimephales promelas*) and rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 36: 164–172.

Various models have been proposed to predict the combined interactive effect on fish of mixtures of poisons from separate toxicities of individual substances. The success of these models was tested, using data describing the lethal and sublethal effects of individual substances or binary mixtures of HCN and Cr(VI), Zn(II), or ammonia to the fathead minnow (*Pimephales promelas*) and rainbow trout (*Salmo gairdneri*). Using the strictly additive toxic unit and additive index approach, it was determined from log-dosage mortality curves that the Zn–HCN and ammonia–HCN mixtures were more acutely toxic and Cr–HCN less toxic than predicted. The concentration and response addition models, which have been proposed for toxicants whose joint action is similar or independent, respectively, could not be used to predict dosage–mortality curves for the HCN mixtures. Linear regression lines representing the growth response of fish to log concentration for toxicants alone and in binary combinations were not significantly different. Thus, for the toxic substances tested, the sublethal joint action of individual toxicants was not predictable from existing models and, in most cases, no interaction was indicated. The interactive nature of toxicants may be a function of the concentrations tested causing different biological processes to be affected (e.g. mortality vs. growth), and therefore different responses to be measured. A need still exists for development of a valid multiple toxicity approach for evaluating and predicting the toxicity of chemical combinations.

Key words: multiple toxicity, binary mixtures, joint action, fish, bioassay, toxic substances

BRODERIUS, S. J., AND L. L. SMITH, JR. 1979. Lethal and sublethal effects of binary mixtures of cyanide and hexavalent chromium, zinc, or ammonia to the fathead minnow (*Pimephales promelas*) and rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 36: 164–172.

Divers modèles ont été proposés dans le but de prédire les effets combinés, sur les poissons, de mélanges de toxiques individuels. Nous avons testé ces modèles avec des données décrivant les effets létaux et sublétaux, sur des têtes-de-boule (*Pimephales promelas*) et des truites arc-en-ciel (*Salmo gairdneri*), de substances individuelles ou de mélanges binaires de HCN et Cr(VI), Zn(II), ou ammoniaque. Utilisant l'approche de l'unité toxique strictement additive et de l'indice additif, nous avons constaté, à partir des courbes de mortalité log-concentration, que les mélanges Zn–HCN et ammoniaque–HCN ont une toxicité plus aiguë et que le mélange Cr–HCN est moins toxique que prévu. Les modèles de concentration et de réaction additive qui ont été proposés pour des toxiques dont l'action conjointe est identique ou indépendante, respectivement, ne purent être utilisés pour prédire les courbes de mortalité-concentration pour les mélanges avec HCN. Les droites de régression représentant la croissance des poissons en réponse au log de la concentration des toxiques seuls ou en combinaisons binaires ne diffèrent pas de façon significative. Ainsi, pour

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les toxiques essayés, l'action conjointe sublétale de toxiques individuels ne peut être prédite avec les modèles existants et, dans la plupart des cas, il ne semble pas y avoir d'interaction. Il se peut que la nature de l'interaction des toxiques soit fonction des concentrations essayées et qui affectent différents processus biologiques (e.g., mortalité vs croissance), de sorte qu'il faille mesurer différentes réactions. On a toujours besoin d'une méthode valide de toxicité multiple pour évaluer et prédire la toxicité de combinaisons de substances chimiques.

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WASTES discharged to natural waters often contain several toxic or potentially toxic substances. However, in most studies of the effects of pollutants, fish have been exposed to single toxicants and relatively few studies have defined the adverse effect of mixtures, especially at sublethal levels. When more than one toxicant is present in the same system, their combined effect may be such that the collective toxicity can be predictable from the separate toxicities of the individual substances. The empirical toxic unit method (reviewed by Sprague 1970) and additive index system (Marking 1977) have been used to evaluate the joint acute toxicity of combined toxicants. These strict summation of toxic fraction methods, while apparently suitable for predicting median lethal tolerance limits of many mixtures, have sometimes not succeeded in predicting median lethal effects and low response percentages (Sprague 1970; Calamair and Marchetti 1973; Eaton 1973; Marking 1977; and Spehar et al. 1978). Some of this disagreement may result from chemical or toxicological interaction and from concentrations of particular mixture components being at levels that exert no toxic action and, thus, according to Lloyd and Orr (1969) should not be expected to contribute to the toxic summation.

Anderson and Weber (1977) have suggested that the toxic unit method could be a simplification of the similar joint action mechanism (i.e. concentration addition) proposed by Bliss (1939). Therefore, linear log-dosage response curves of individuals exposed to separate toxicants that are similar chemically or act similarly would be essentially parallel (i.e. relative susceptibilities of individuals would be completely and positively correlated). In such cases Anderson and Weber (1977) have provided a procedure to predict a dose–response curve for the mixture. This type of interaction has been observed for some toxicants, but other toxicologists (Plackett and Hewlett 1952; Hewlett and Plackett 1959; Ariëns and Simonis 1961; and Casarett 1975) have pointed out that parallelism is not a requirement for similar joint action. Bliss (1939) described another additive mechanism that he denoted as independent joint action (i.e. response addition, Anderson and Weber 1977). Plackett and Hewlett (1948, 1952) and Hewlett and Plackett (1950) described mathematically this mode of joint action, where each toxic component of a mixture acts upon different biological systems within an organism, with each toxicant contributing to a common response only if its concentration exceeds a certain tolerance threshold.

There is limited and contradictory evidence for the interaction of sublethal concentrations of toxic components in mixtures. Eaton (1973) was able to demonstrate some interaction, but not strict summation, of individual toxicant chronic responses by fathead minnows exposed concurrently to copper, cadmium, and zinc. Sprague et al. (1965) observed that sublethal levels of copper and zinc together produced an additive joint avoidance reaction by Atlantic salmon. Negilski (1973) reported that a combination of cyanide, pentachlorophenol, and zinc produced a greater than additive effect on the growth and production of juvenile chinook salmon following exposure to sublethal levels in laboratory model streams. Anderson and Weber (1977) suggested that Negilski's results could be interpreted as representing a form of response addition. Muska (1978) demonstrated that the concentration addition model adequately predicted the effects of a copper–nickel, but not a dieldrin–nickel mixture on certain measurements of growth for juvenile guppies. Spehar et al. (1978) observed that mixtures of cadmium and zinc did not act additively in chronic tests at sublethal levels but a joint action of the metals was indicated. Therefore, it seems that in certain instances toxicants can combine to produce enhanced sublethal effects in fish.

The proposed models for assessing the joint effects of poisons to fish have not been adequately tested. Because it is important to be able to predict toxicity in natural waters from a knowledge of individual toxic constituents, the purpose of this investigation was to empirically examine the validity of various methods for predicting joint toxicity of binary mixtures of HCN with Cr(VI), Zn(II), or ammonia from lethal and sublethal responses of fish to individual toxic components in the mixtures.

Materials and Methods

TEST WATER AND FISH

The well water used in all tests had a total alkalinity and hardness of 235 and 220 mg/L as $CaCO_3$, respectively. A comprehensive analysis of this water was reported by Smith et al. (1976).

Newly hatched larvae or juvenile fathead minnows (*Pimephales promelas*) and fry or juvenile rainbow trout (*Salmo gairdneri*) were used as test organisms. The juvenile minnows (cultured in the laboratory from a strain obtained from the U.S. Environmental Protection Agency's Environmental Research Laboratory, Duluth, Minn.) were approximately 8 wk old at testing, had mean total lengths of 19 mm, and mean wet weights among survivors of 0.079 g. The sensitivity of different lots of juvenile minnows to any specific toxicant was demonstrated by periodic acute toxicity tests to be quite uniform throughout the experimental period.

Two lots of eyed rainbow trout embryos from eggs spawned in late April 1976 and 1977 were obtained in early July 1976 and 1977, respectively, from the French River State Fish Hatchery, French River, Minn. The water temperature at collection and during subsequent holding and testing of the trout was 10°C. The trout reared from the 1976 stock were approximately 22 wk old when used for lethal tests, had mean total lengths of 53 mm, and mean wet weights among survivors of 1.48 g. Fry reared from the 1977 stock were used in the sublethal tests.

Testing Conditions

The lethal and sublethal tests were conducted in identical diluters with cyclic water delivery and toxicant introduction systems similar to those described by Brungs and Mount (1970) and Mount and Warner (1965), respectively. Each experimental glass chamber measured 25 × 50 × 30 cm high and contained 35 L of test solution exchanged at the rate of 250 mL/min, affording 95% replacement in about 7 h (Sprague 1969).

Water temperature was maintained within ± 0.1°C (SD) at 25°C for all tests with fathead minnows and at 10°C for rainbow trout. Aeration in the head water reservoir feeding the diluters maintained dissolved oxygen concentrations in the test chambers at approximately 80% of saturation. A sulfuric acid solution was automatically dispensed into the reservoir to control the pH of the experimental water at near 7.80 for tests with fathead minnows. In tests with rainbow trout the pH was not controlled but remained constant at near 7.95. The test chambers were illuminated for 16 h daily with two 40-W fluorescent tubes 45 cm above the chambers. Stock toxicant solutions of NaCN, $Na_2Cr_2O_7 \cdot 2H_2O$, $ZnSO_4 \cdot 7H_2O$, and NH_4Cl were prepared with reagent grade chemicals and deionized water. Tests with ammonia were conducted at 10°C to retard nitrification and prevent the buildup of adverse nitrite levels in the test chambers.

Chemical Analysis

Water temperature, dissolved oxygen, pH, and toxicant concentrations in each test chamber were measured daily during each lethal test and 3 times per week during the sublethal tests. Determinations for each test were averaged and used as the experimental parameters. Free cyanide concentrations were determined by the pyridine–pyrazolone colorimetric method (APHA et al. 1971). Free ammonia, Cr(VI), and Zn(II) concentrations were determined by the direct nesslerization, diphenylcarbazide, and zincon colorimetric procedures, respectively (APHA et al. 1975). The concentration of molecular HCN or NH_3 was calculated for each cyanide (i.e. $HCN + CN^-$) or ammonia (i.e. $NH_3 + NH_4^+$) determination using the daily pH and temperature measurements and the pK equilibrium constants (i.e. $-\log K_a$) calculated from the equations

$$pK_{HCN} = 3.658 + 1662/T \text{ (S. Broderius unpublished data)}$$

$$pK_{NH_3} = 0.09018 + 2729.92/T \text{ (Emerson et al. 1975)}$$

where T is temperature in degrees Kelvin. The ratio $[HCN]/[HCN + CN^-]$ or $[NH_3]/[NH_3 + NH_4^+]$ is equal to the factor $1/(1 + 10^{pH - pK_a})$ or $1/(1 + 10^{pK_a - pH})$, respectively.

Lethal Tests

The 96-h lethal tests were conducted in diluters each including one control and four treatments in a geometric series. Twenty fathead minnows or 10 rainbow trout juveniles were randomly distributed into each treatment and control chamber. The fish were acclimated to the test chambers for 2–3 d before introduction of the toxicants. During the 24-h period preceding and throughout a lethal test the fish were not fed. In each binary experiment the single toxicant tests were completed before the joint toxicant tests. Since all the lethal tests for each pair of toxicants were completed within a few weeks, changes in fish susceptibility within a test period for two toxicants were assumed to be nonsignificant.

Percentage fish mortality after 96-h exposure to individual toxicants and to binary mixtures containing fixed proportions of the constituents was used as the response criterion. Estimates of the concentration of toxicants most likely to cause 50% mortality (LC50) and toxicity curve slopes after 96-h exposure were made by a log-probit analysis computer program (Dixon 1973). All the data from tests conducted for specific toxicants or for their mixtures were composited for probit analysis. The 95% confidence limits for LC50 values were also computed (Litchfield and Wilcoxon 1949; Finney 1971).

Sublethal Tests

Growth for 30 d of groups of fathead minnows or rainbow trout fed an unrestricted ration was used as the quantitative response to sublethal joint action of binary toxicant mixtures. Graded dose–response curves were determined by measuring terminal mean dry weight for fish exposed to individual toxicant and selected mixture concentrations ranging from levels causing small or no change in growth to those approaching the incipient lethal level. Growth tests were begun by randomly adding 50 1-d-old fathead minnow larvae or 20 4-wk-old rainbow trout fry to each of 21 treatment and 4 control test chambers. From a subsample of 32 individuals, it was determined that the trout fry initially were 28.5 ± 2.2 mm in mean total length (±SD), and 0.180 ± 0.059 g and 0.0278 g in mean wet and dry weight, respectively. The treatments consisted of seven independent cyanide and seven second toxicant levels, and seven binary mixtures for each test.

Through the 1st wk of exposure, the fathead minnow larvae were fed a fine, hard-boiled egg yolk suspension 3 times daily. Beginning the 2nd wk a supplement of finely ground Glencoe granules was added twice daily for the remainder of the test. Beginning the 3rd wk, finely chopped lettuce was added to the egg yolk suspension, and newly hatched brine shrimp nauplii (*Artemia*) were fed twice daily. During the first 2 wk of the trout tests fry were

fed twice daily with ground Glencoe starter and 3 times daily with newly hatched brine shrimp. Beginning the 3rd wk, Glencoe No. 1 and Oregon Moist Pellets were fed 3 times per day and brine shrimp twice daily. Food slightly in excess of what the fish consumed in 5 min was added to each chamber. At the termination of each test surviving fish were measured and weighed individually. Mean weights of fish that had been dried for 24 h at 105°C were determined for each treatment. Mean dry weights were about 19% of mean wet weights.

Linear and multiple regression analyses were applied to growth data to determine whether the slopes and intercepts of the relationships defining mean dry weight vs. log toxicant concentration were the same for a specific toxicant by itself and in a binary mixture.

Mixture Models

The calculation of the combined effect of mixtures of toxicants by the toxic unit approach has been outlined by Sprague (1970). This method assumes that a mixture should elicit a particular toxic effect, such as a median lethal concentration, when the sum of the concentration of the component toxicants, each expressed as a proportion of the individual toxicants acutely lethal concentration, equals unity. The proportional toxicity of each poison in a mixture is obtained by dividing the actual concentration in solution by its calculated 96-h LC50. These values obtained for the two toxicants in a binary mixture are then summed to give the proportion of the 96-h LC50 in the mixture. The concentrations selected for testing were usually such that each poison theoretically contributed an equal proportion of toxic units to the mixture. The toxic unit–percentage mortality relationships were analyzed by the computer program as outlined above (Dixon 1973).

The toxic unit (TU) or sum of biological activity index of joint toxicity can be expressed as an index of additive toxicity linearly distributed about zero, which represents simple additive toxicity. This additive index as proposed by Markng (1977) is equal to $[(1/\mathrm{TU}) - 1.0]$ for $\mathrm{TU} \leq 1.0$ and $[\mathrm{TU}(-1) + 1.0]$ for $\mathrm{TU} \geq 1.0$. Thus toxicity greater than and less than simple additive toxicity is represented by positive and negative index values, respectively. The magnification factor is obtained by adding one to the numerical index value for greater than additive toxicity and taking the reciprocal of the numerical index value for less than additive toxicity. The significance of deviation from zero is determined by substituting selected values of the 95% confidence limits for the calculated toxic units into the appropriate additive index formula to establish the deviation in the additive index range. Simple additive toxicity is assumed whenever an index range overlaps zero.

If the linear toxicant response curves of individuals exposed to two toxicants are not parallel or if the modes of toxic action of the two constituents are known to be different for toxicants that have similar slopes, then it is possible that the separate responses of organisms to the individual concentrations of each toxicant in a mixture can be summed. The response to be expected in such a binary mixture exerting independent joint action (Bliss 1939) or response addition (Anderson and Weber 1977) depends upon the correlation between the susceptibilities of the individual organisms to each toxicant. According to Plackett and Hewlett's (1948, 1952) mathematical models, if the correlation of individual tolerances is completely negative ($r = -1$) so that the proportion of individuals most susceptible to one toxicant alone (P_1) are least susceptible to the other (P_2), then the proportion of individuals expected to respond to the mixture (P_m) can be represented by

$$P_m = P_1 + P_2 \text{ if } (P_1 + P_2 \leq 1) \tag{1}$$

With no correlation in susceptibility ($r = 0$) the relationship is expressed by

$$P_m = P_1 + P_2 (1 - P_1) \tag{2}$$

If there was a complete and positive correlation ($r = 1$) so that individuals most susceptible to the first toxicant would be correspondingly susceptible to the second, then

$$P_m = P_1 \tag{3}$$

where P_1 is the response to the most toxic constituent in the mixture.

Results

The results of lethal tests using individual toxicants and binary mixtures are summarized in Tables 1 and 2. Probit analysis of data from binary mixture tests, using the log summation of the individual concentrations of toxicants in each treatment mixture expressed as proportions of their 96-h LC50 values, as the dose measurement, generally gave 96-h LC50 toxic unit values of slightly <1.0 (Table 2). The Cr–HCN mixture was the exception with a 96-h LC50 toxic unit value of 1.31. Since the 95% confidence limits for the toxic unit values do not bracket 1.0 in any case, nor does the additive index range overlap zero, the estimated values for the 96-h LC50 concentrations differ significantly from that expected from the simple additive toxic unit hypothesis. Therefore, the acute lethal toxicities of various binary mixtures of HCN and Cr(VI), Zn(II), or ammonia could not be predicted for the ratios tested by summing the fractional toxicities of the particular poisons present in each mixture. In two of the three cases, the mixtures exerted a supraadditive effect (i.e. toxicity was greater than predicted) with the Zn–HCN and ammonia–HCN mixtures being 1.4 and 1.2 times as toxic as predicted, respectively (Marking 1977). The exception was Cr(VI) whose interaction with HCN seems to be of the infraadditive type since the mixture was 0.76 times as toxic as predicted. It appears that for each fish species the dose–response regression slope value (β) is nearly the same for HCN alone as for mixture tests analyzed by the toxic unit approach (Tables 1 and 2), even when the individual toxicants differ both in potency and in the slopes of their dosage–mortality curves. This result suggests that for both species HCN exerts its toxic action in a similar manner and is the factor that defines the slopes of the mixture response curves.

For most of the individual toxicant and mixture tests a median lethal threshold concentration could be determined in 96-h exposure. However, for Cr(VI) alone additional mortality of fathead minnows occurred beyond this period. The 10-, 20-, and 30-d LC50 and 95%

TABLE 1. Dose–response parameters and lethal concentrations for acute toxicity bioassays of individual toxicants; 95% confidence limits in parentheses.

Toxicant	Dose–response regression analysis[a] Treatments[b]	Intercept α	Slope β	96-h LC50 (mg/L)
		Fathead minnows		
HCN	36	16.75	13.01	0.125 (0.120 – 0.130)
Cr(VI)	30	−2.787	5.119	33.2 (31.1 – 35.4)
Zn(II)	32	2.590	5.776	2.61 (2.49 – 2.75)
		Rainbow trout		
HCN	15	33.63	23.04	0.0572 (0.0558 – 0.0586)
NH_3	23	6.325	8.460	0.697 (0.629 – 0.773)
Total ammonia-N	23	−9.069	9.063	35.7 (32.5 – 39.1)

[a]For equation $Y_t = \alpha + \beta (\log X_t)$ when Y_t is the predicted percentage mortality as probits and X_t is log toxicant concentration in milligrams per litre (Dixon 1973).
[b]Number of toxicant concentrations in the regression analysis.

TABLE 2. Dose–response parameters, and toxic unit and additivity indices for acute toxicity bioassays of binary mixtures; 95% confidence limits in parentheses.

Toxicant	Toxicant concn ratios, tox./HCN[a] Aim	Determined	Dose–response regression analysis[b] Treatments[c]	Intercept α	Slope β	96-h LC50 (toxic units)	Additive index and range
				Fathead minnows			
Cr(VI)–HCN	266	235 ± 20	20	3.906	9.277	1.31 (1.22 – 1.41)	−0.312 (−0.408 to −0.222)
Zn(II)–HCN (1)	20.9	14.3 ± 1.0	10	7.008	13.19	0.704 (0.650 – 0.763)	0.420 (0.311 to 0.538)
Zn(II)–HCN (2)	20.9	23.2 ± 2.7	13	6.966	13.87	0.721 (0.692 – 0.752)	0.387 (0.330 to 0.445)
				Rainbow trout			
NH_3–HCN	12.2	12.4 ± 1.1	10	6.401	21.31	0.859 (0.824 – 0.897)	0.164 (0.115 to 0.214)
(Total ammonia-N)–HCN	624	594 ± 38	10	6.668	20.08	0.826 (0.790 – 0.864)	0.211 (0.157 to 0.266)

[a]Ratio of individual toxicant concentrations in different treatments of mixture tests. Aim values are for equal proportions of toxic units.
[b]For equation $Y_t = \alpha + \beta (\log X_t)$ when Y_t is the predicted percentage mortality as probits and X_t is log toxicant concentration in toxic units (Dixon 1973).
[c]Number of toxicant concentrations in the regression analysis.

confidence limits, determined under similar test conditions as reported for 96-h tests with Cr(VI) (Table 1), were 12.4 (11.0-14.0), 5.99 (5.14-6.96), and 4.36 (3.85-4.92) mg/L as Cr(VI), respectively.

The slopes of the dose–response lines for each component of a mixture were significantly different when tested separately. Parallelism between response curves is apparently a prerequisite to concentration addition as defined by Anderson and Weber (1977), but not for response addition. Therefore, it is possible that this latter model, for toxicants that are thought to differ in their action on biological systems, could predict responses comparable to those observed in the binary mixtures. When this approach was used to analyze the data, it was determined that in every case the expected mortality was significantly less using a chi-square test ($P = 0.05$) than was experimentally obtained. Using the dose–response regression lines for the individual toxicants and assuming the response addition model with no correlation in susceptibility to the two toxicants (equation 2), a mortality of about 20% was predicted among test fish exposed to the Cr–HCN mixture which, in fact, killed about 50%. For the Zn–HCN and ammonia–HCN mixtures in which the observed mortality was about 50%, the predicted mortality was <1.0%.

Bliss (1939) has pointed out that for individual toxicants that act independently and have dosage–mortality lines with different slopes, the response to a binary mixture should have some compound discontinuity consisting essentially of two straight line seg-

ments. The slopes of these rectilinear segments should also approach the slopes of the regression lines for the individual constituents. This result was not observed for the mixtures tested since in all cases a single straight line adequately defined the dosage–mortality relations. It was also observed that either toxicant could contribute to the toxicity of the mixtures tested even when individually present at a concentration that would result in no mortality if the other was absent. However, responses to toxicants acting upon different biological systems are predicted to be additive only if each toxicant in a mixture is present at a concentration above its respective threshold level. Therefore, we conclude that the response and concentration addition models (Anderson and Weber 1977) cannot be used to predict dosage–mortality curves for the mixtures studied.

The results of 30-d growth tests with fathead minnows and rainbow trout exposed to individual toxicants or binary mixture solutions are summarized in Fig. 1. Terminal survival of fathead minnows in the controls was about 60% of that initially added as fry. In tests with HCN and Cr(VI) there was no effect of these toxicants on survival when compared with the controls. However, in tests with Zn(II) at 0.76 and 0.84 mg/L, and those with a mixture of HCN and Zn(II) at 0.045 and 0.62, and 0.057 and 0.86 mg/L, respectively, survival was about 65% and 40% of that in the controls, respectively. Rainbow trout were markedly affected by 0.044 mg/L HCN with survival after 30-d exposure being 30% of that in the controls which had 100% survival. Ammonia at 0.32 mg/L, and a mixture of HCN and NH_3 at 0.028 and 0.19 mg/L also reduced survival to 70% of that in the controls.

The maximum percentage reduction in mean dry weight for concentrations approaching the incipient lethal level were all about 50%, with the greatest being 79% for 3.06 mg/L of Cr(VI) alone. Since F tests showed that there was no significant difference ($P < 0.05$) between appropriately paired linear regression lines defining the relationship of weight to log concentration, no interactive sublethal effect on growth could be demonstrated for the toxicants tested. This similarity of regression lines can be seen in Fig. 1 by comparing the intercept and slope parameters for individual toxicants with the parameters for those toxicants when in binary mixtures. HCN in combination with ammonia was the only mixture that seemed to demonstrate some sublethal interactive effect on growth of rainbow trout. When terminal mean dry weight was regressed on log HCN, NH_3, or total ammonia-N concentrations, the weight–concentration relationship for a specific toxicant in a binary mixture indicated in all cases a greater effect than that determined for the corresponding toxicant when tested alone (Fig. 1). The linear regression relationship for terminal mean dry weight of rainbow trout in milligrams (Y) as a function of log total ammonia-N in milligrams per litre (X) can be represented by $Y = 251 - 132 \log X$ ($r = -0.926$) for total ammonia-N alone and by $Y = 274 - 171 \log X$ ($r = -0.975$) when in combination with HCN. The only regression relationships that were statistically different at an acceptable level of significance ($F_{2,10} = 3.67$, $P < 0.063$) were those calculated for the effect of HCN alone compared to when HCN was in combination with ammonia. Therefore, the sublethal stress imposed on fish in most binary mixtures studied disrupted normal physiological processes so that the relationships between concentration and reduction in growth were essentially equal to those when the toxicants were present alone. The adverse joint action of individual toxicants on growth were not predictable according to certain proposed models.

Discussion

A thorough understanding of chemical reactions of constituents in a mixture is necessary for prediction of joint effects. Where toxicants chemically combine, prediction of joint toxicity may be most difficult. Chromium and zinc can combine with cyanide to form coordination compounds. However, calculations from stability constants showed that under the conditions prevailing in the mixture solutions, <1.0% of the cyanide, and even smaller percentages of the metals, would be present as complex ions (Doudoroff 1976; Ford-Smith 1964). Therefore, the toxicants in each of the binary mixtures studied should be in essentially the same form as they were in the solutions where their toxicities were individually determined.

The physiological basis for joint interaction of toxicants on fish has received some attention in recent years. As pointed out by Marking (1977), projected theories for the mechanisms of increased toxicity in mixtures include increase in rate of uptake, formation of toxic metabolites, reduction of excretion, alteration of distribution, and inhibition of detoxification. Lloyd and Swift (1976) studied whether the increased water uptake by fish exposed to high ammonia concentrations was accompanied by an increased rate of phenol uptake in ammonia–phenol mixtures. They concluded that the rate of uptake and concentration of phenol in fish muscle is not influenced by elevated ambient ammonia levels nor is the urine flow rate increased for rainbow trout exposed to ammonia–phenol solutions. Therefore, no physiological bases could be found to account for the empirical, additive toxic action of ammonia and phenol. The effects measured in the binary mixture were those corresponding to either ammonia or phenol alone and the presence of one did not appear to enhance the effect of the other.

In the present study, it was thought that the mode of toxic action of the poisons investigated was dissimilar. However, lethal effects were apparently not independent and each toxicant in our binary mixtures did not act to produce its own separate stress which, in the prediction of joint toxicity, could be summed according to existing

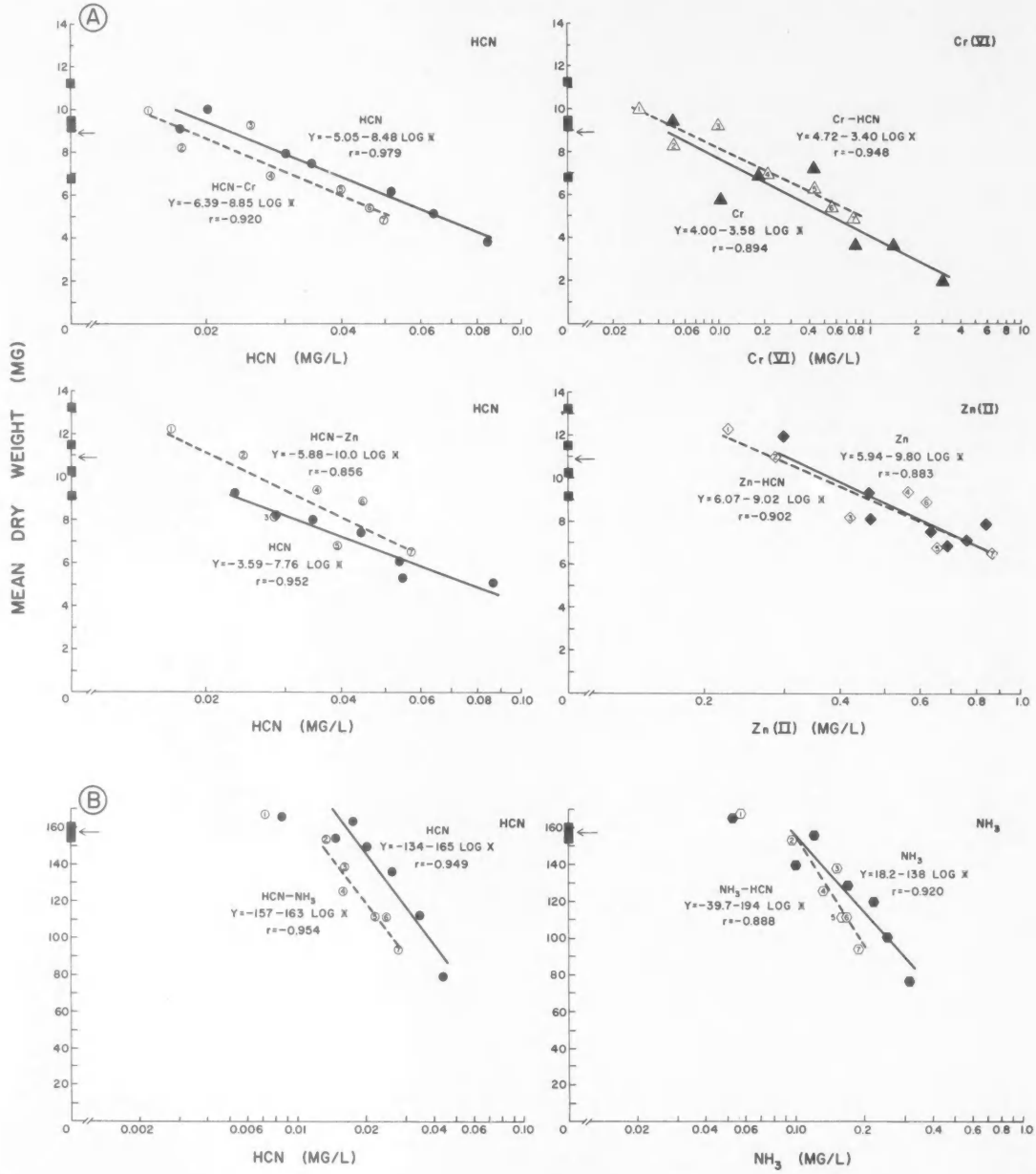

FIG. 1. Growth of fathead minnows (A) or rainbow trout (B) in 30 d when exposed to individual (closed symbols and solid lines) or binary mixture solutions (open symbols and dashed lines) as expressed by linear regression analysis of the toxicant dose–response curves relating terminal mean dry weight in milligrams (Y) as a function of log toxicant concentration in milligrams per litre (X). Specific mixture test solutions are numerically indicated with the weighted mean dry weight for the controls, based on the number of survivors in each replicate, marked by an arrow.

multiple toxicity models. The interactive nature of toxicants studied was also a function of the particular response measured. The type of joint toxicity observed for fish exposed to lethal concentrations of mixtures did not occur when they were subjected to sublethal levels of the same toxicants. The complexity and interdependency of physiological systems, which can result in partial similarity of toxic actions, strongly suggests that special types of interaction represented by concentration and response addition may be appropriate only occasionally. More general theories for the interpretation of quantal response data for mixtures, such as proposed by Hewlett and Plackett (1959) and Plackett and Hewlett (1967), may be more suitable. These theories permit any degree of correlation of tolerances and any degree of similarity between the biochemical or physiological action of toxicants in predicting quantal responses to toxicant mixtures. Since it has not been established whether the adverse effects of toxicant mixtures on fish can be predicted from individual toxicant effects, a need still exists for development of a valid multiple toxicity approach to evaluate the toxicity of chemical combinations. Such an approach will most likely include a better understanding of the mode of action for the toxicants of interest.

Acknowledgments

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Twilight Activities of Yellow Perch, *Perca flavescens*

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HELFMAN, G. S. 1979. Twilight activities of yellow perch, *Perca flavescens*. J. Fish. Res. Board Can. 36: 173–179.

Underwater observations were made on the diel activity patterns of yellow perch (*Perca flavescens*) in Cazenovia Lake, Madison County, New York (42°56′N, 75°52′W). Perch underwent a characteristic progression of activities during dusk at relatively predictable light levels and times. Patterns within the progression included increased swimming, last feed, group breakup, slowdown, and final stop. Activity at sunrise was the reverse of the sunset sequence except that some activities occurred at lower light levels than did the analagous activities in the evening. Differences exist in the twilight activity patterns of perch in different lakes; within a lake, both ontogenetic and subpopulation differences are also observed. These differences may be responses to ecological conditions, such as relative predation levels, or they may reflect the extreme plasticity of the species.

Key words: behavioral ecology, changeover, diel, diurnal, fish, light, nocturnal, ontogeny, plasticity, temperate lake

HELFMAN, G. S. 1979. Twilight activities of yellow perch, *Perca flavescens*. J. Fish. Res. Board Can. 36: 173–179.

Nous avons observé sous l'eau les activités nycthémérales de la perchaude (*Perca flavescens*) dans le lac Cazenovia, Cté de Madison, New York (42°56′N, 75°52′O). Les perchaudes exhibent une progression caractéristique de leurs activités au crépuscule à des niveaux de clarté et des heures relativement prévisibles. Parmi les activités comprises dans cette progression, on note : nage plus active, dernier repas, éparpillement des groupes, ralentissement et finalement arrêt. Au lever du soleil, l'activité est à l'inverse de la séquence observée au coucher du soleil, sauf que certaines activités ont lieu à des intensités lumineuses moindres que durant les activités analogues du soir. Il y a des différences dans le comportement crépusculaire des perchaudes de différents lacs; on observe aussi, dans un même lac, des différences à la fois ontogénétiques et de sous-populations. Il se peut que ces différences soient des réactions aux conditions écologiques, telles que les niveaux de prédation relatifs, ou encore qu'elles reflètent l'extrême plasticité de l'espèce.

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MOST organisms engage in different activities by day and by night. Twilight is the period of transition between these modes; it is a time when both light quantity and quality are changing and it exerts a strong selective force on the visual and behavioral adaptations of many animals (McFarland and Munz 1976; Munz and McFarland 1977). Interest in the twilight activities of fishes has been largely restricted to tropical marine communities and temperate marine species (reviewed by Helfman 1978 and Blaxter 1976, respectively). The twilight activities of temperate freshwater fishes have received comparatively little attention, particularly with respect to direct behavioral observations. Hasler and Bardach (1949) and Hasler and Villemonte (1953) conducted the pioneer work, making limited in situ observations of yellow perch (*Perca flavescens*) at twilight in Wisconsin. More recently, Emery (1973) briefly described the evening behavior of 20 species of Ontario lake fishes, and Ryder (1977) gave detailed accounts of crepuscular feeding activity in walleye (*Stizostedion vitreum vitreum*). The present study is part of a larger investigation of twilight activities in lake fishes (Helfman 1979). Particular attention was given yellow perch as it was the only abundant species in the study area for which at least preliminary comparative data were available (Hasler and Bardach 1949; Hasler and Villemonte 1953). In this paper I will briefly describe the daytime and nighttime activity modes of the yellow perch, detail the sequence of activities during morning and evening twilight, discuss differences between young-of-the-year and older fish, and compare the twilight behavior of perch in different North American lakes.

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Materials and Methods

Observations were made in a 1.3-ha bay in Cazenovia Lake, Madison Co., N.Y. (42°56′N, 75°52′W). Cazenovia Lake is a 475-ha, mesotrophic, dimictic lake with a maximum depth of 14 m. The fish community is typical of small lakes of the region and consists of 18 common species dominated by centrarchids (6 species) and percids (3 species). The lake is typically ice-free from April to December. Water clarity is usually fair, with daytime horizontal visibility (Secchi disc) averaging 5 m during the summer months.

All observations were made during approximately 110 h of skin or scuba diving between 1974 and 1977. Data are based on 31 dusk and 13 dawn periods, plus numerous daytime observations. Most data are from fish inhabiting the shallow fringes of the lake at depths of <2.5 m. Data were obtained from individuals chosen randomly or from fish tagged for individual recognition with 2–3-cm color-coded Tygon tubing (modified from White and Beamish 1972). Five tagged individuals were followed through most or all of an evening changeover sequence, while 29 other individuals were observed through parts of an evening sequence. Two tagged fish were observed through morning twilight, with additional data drawn from 17 nontagged fish. Although the size of an observed fish was recorded to the nearest centimetre total length, the data are presented for two general size-classes, young-of-the-year (YOY, 1–4 cm) and post-young-of-the-year (PYOY, >4 cm).

Times of events were recorded to the nearest minute. Times of sunrise, sunset, and twilight were calculated from an almanac (Anon. 1974), corrected for longitude and latitude. Time relative to sunset and sunrise is reported as percent of astronomical twilight, to allow for variations in astronomical twilight length from 96 to 144 min during the study months. Hence twilight has been standardized to 100 min for all observational periods and a time value such as "SS + 10%" could occur between 9 and 20 min after sunset.

Light was measured with a Gossen Lunapro photographic light meter in an Ikelite underwater housing. All measurements were of downwelling radiance (i.e. with the meter pointed at the water surface) and were recorded at or near the place and depth of the behavioral observation. A conversion from Lunapro Units (LPU) to approximate irradiance values can be obtained by lux = (0.002) [2.275$^{(LPU)}$]. Additional details of light measurement are given by Helfman (1979).

Results

Daytime Mode

The activities of yellow perch during daylight have been described previously (e.g. Nursall and Pinsent 1969; Nursall 1973; Helfman 1979) and are mentioned only briefly here as background for comparing twilight events.

Yellow perch in summertime occurred in four major regions of the study area during the day. Schools of YOY were largely restricted to vegetation in 1 m or less until approximately 2 mo after hatching; solitary YOY perch were seldom encountered (see Kelso and Ward 1977 for diel movements of perch fry). PYOY could be subdivided into three size-groups. Small juveniles 7–10 cm long were restricted to depths of <2 m and generally occurred in groups of <10 individuals. Fish 10–20 cm long formed small foraging schools in 1–2 m, while larger schools foraged below the littoral zone in approximately 5–7 m of water. Fish >20 cm were usually solitary and were most common at depths of 2–4 m in a heavily vegetated drop-off region.

Evening Sequence

The behavior of yellow perch in the evening was characterized by a sequence of group and individual activities that represented a transition from daytime activity to nighttime inactivity (Fig. 1).

Among PYOY fish, as sunset approached and light dimmed, individuals and schools increased swimming activity and swam with fewer turns and stops. If observed from one point, this activity consisted of an increase in the number of individuals passing the point, i.e. a flux of individuals. The evening flux was recorded as an increase in distance traveled by a followed individual (six evenings, quantified on four evenings: $\bar{x}$ speed during 10 min before flux ± SD = 0.8 m/min ± 0.6; during flux = 8.7 m/min ± 3.9), or as an increase in the number of perch passing a fixed point (four evenings: $\bar{x}$ number during prior 10 min = 0 fish/min; during flux 1.7/min ± 1.0). The fish usually swam slightly higher above the bottom than during normal daytime activities. Most movements were offshore towards the thicker vegetation and deeper water (four observations), although one inshore and one longshore movement were also seen. On one occasion a perch moved to deeper water and then returned to the area where the flux had begun. Four individuals showed no evidence of an evening flux.

Once, a YOY school moved 20 m from the shoreline when flux might have been expected. On three other evenings, no increased activity was seen in YOY perch.

During or shortly after flux, perch fed for the last time. In PYOY, last feed usually occurred shortly after sunset; in YOY, two of three last feeds occurred just before sunset. Light levels were still relatively high (Fig. 1) and the fish quite active. Although feeding ceased, other maintenance-type activities, such as posing for cleaning by bluegill (e.g. Sulak 1975) and chafing against hard substrate (e.g. C. R. Patmont and G. S. Helfman unpublished data), continued or increased.

As light levels continued to fall, schools of PYOY fragmented into progressively smaller groups until fish were swimming in pairs or triplets, then as solitary individuals. Group breakup in YOY followed slowdown as described below.

Shortly after last group among PYOY fish, individual fish displayed a characteristic slowdown sequence. This

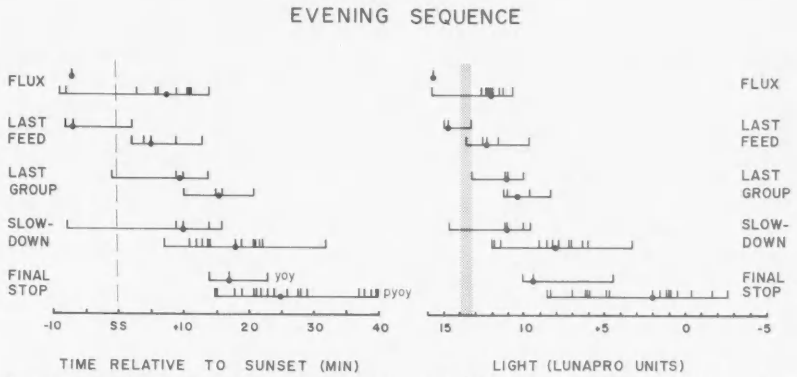

FIG. 1. Evening sequence of activities in young-of-the-year (YOY) and post-young-of-the-year (PYOY) yellow perch in Cazenovia Lake. Each activity is represented by two horizontal lines, with the data for YOY above those for PYOY. Each vertical mark represents one observation, while the black dots are the median values for each activity (medians rather than means are used due to a high degree of individual variation). On the left is time relative to sunset, given as percent twilight, i.e. standardized to a 100-min astronomical twilight; minus (−) values are before sunset, plus (+) values are after sunset. On the right are light levels in Gossen Lunapro units (for conversion to lux, see Materials and Methods). Light values less than zero are based on an extrapolation of the light curve obtained for the evening in question and are therefore approximate. The stippled region shows the usual light levels at sunset.

sequence usually began with a marked slowing in speed as the fish swam within 30 cm of the bottom. Most fish then stopped for increasing periods, typically first for 1–2 min, then 3–9 min. During these stops the fish usually hovered 10–30 cm above the bottom. Some individuals stopped without initially slowing down, while others came to a final stop after slowing without intermediate stops.

Fish going through the slowdown phase typically spent between 5 and 10 inactive minutes ($\bar{x} = 6.1 \pm 1.4$ min, $n = 6$ fish) before the final stop. The complete slowdown phase lasted from 0 to 39 min ($\bar{x} = 18.7 \pm 23.6$ min, $n = 6$). During this period, fish showed a decreasing sensitivity to visual and mechanical stimuli. Illuminating a perch momentarily with a flashlight during the initial phase of slowdown usually caused it to swim off rapidly; it responded similarly to the approach of a diver or to the movements of other fish. As the slowdown progressed, i.e. as stops increased in length, the fish became less sensitive to the flashlight beam and other stimuli. In essence, the fish was changing to the nocturnal resting mode.

YOY perch behaved similarly, except that the schools slowed down collectively before breaking up. On two occasions schools slowed or stopped initially as a unit. The fish then disbanded and individuals were soon scattered and resting on the bottom or in vegetation.

After the slowdown sequence, fish rested just above or slightly touching the bottom (if in an area of short vegetation or over barren substrate), or in taller vegetation at varying angles and heights above the bottom. Final stop was determined by flashing the light briefly at 1-min intervals for 5–10 min after the last stop. On several occasions, I returned to the location of the final stop 15–30 min later to confirm that a fish was in fact resting there, which it usually was.

NIGHTTIME MODE

PYOY perch showed a weak affinity for structure at night. Some sought shelter in vegetation, but the majority rested in exposed areas of relatively short or no vegetation, both in the shallows and deeper regions. The tendency to contact plants was strongest in YOY and in PYOY individuals longer than 20 cm (see Stamm 1977, p. 110–111 for photographs of resting perch). The smallest and largest perch were seldom found in exposed shallow areas, but were more common on the barren sublittoral zone at night.

PYOY moved little during the night if not disturbed (except on the night of April 26, 1977, when active spawning aggregations were encountered in 1 m of water). Shining the diving light for several seconds often caused resting fish to move, which made it difficult to determine how sedentary an individual was. However, on two nights I successfully made periodic checks on tagged individuals. These fish moved a total of 1 and 3 m, respectively, during the night.

The body coloration of resting PYOY was different from that of active fish during the daytime. The vertical bars darkened and the interspaces became tinged with green at night.

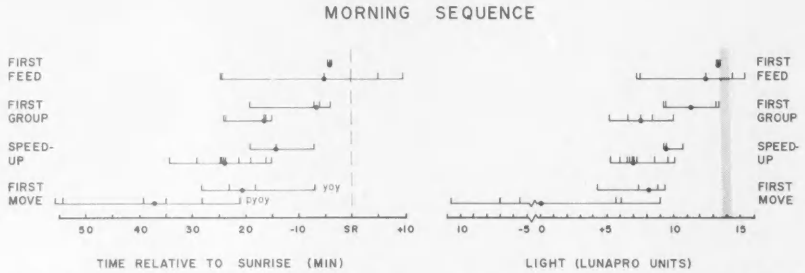

FIG. 2. Morning sequence of activities in young-of-the-year (YOY) and post-young-of-the-year (PYOY) yellow perch in Cazenovia Lake. See Fig. 1 for explanation.

MORNING SEQUENCE

The sequence of events among PYOY fish in the morning (Fig. 2) was roughly the reverse of that observed during dusk, with the major exception of a lack of a noticeable flux. It is possible that morning flux occurred simultaneously with the other events as the fish changed to the daytime mode.

Fish remained motionless until light intensity reached $\approx 2 \times 10^{-3}$ lx. Initially, a fish rotated slowly, rose slightly off the bottom, and moved a short distance. This first move and stop was followed by longer moves and shorter stops, during which the fish yawned frequently. Finally the fish increased its speed and moved continuously. Shortly after the continuous movement began, individuals swam with other fish, small groups merged into larger groups, which usually (three of five observations) began feeding just before sunrise. Feeding activity peaked and then slowed.

YOY fish behaved similarly, except that groups increased in size with little intermediate fragmenting and rejoining. All individuals appeared to form groups as soon as they encountered other individuals, i.e. there was less lag between first move and group formation than in larger fish. First feed occurred shortly before sunrise.

Discussion

COMPARISONS BETWEEN POPULATIONS

Diel activity patterns — Both yellow and Eurasian perch are diurnally active and nocturnally inactive, with presunset and postsunrise activity peaks (e.g. Alabaster and Robertson 1961; MacLean and Magnuson 1977). These peaks often correspond to onshore migrations in the evening and offshore migrations in the morning. In several studies, the authors reported that schools break up in the evening and reform in the morning, that migrations occur from daytime feeding to nighttime resting areas, and that fish cease feeding during the migration (Pearse and Achtenberg 1920; Scott 1955; Craig 1977; see also reviews in Carlander and Cleary 1949; Hasler 1963; Hergenrader and Hasler 1966; and Thorpe 1977 a, b). Migrations in the other lakes occur at about the same time or light levels as did flux in Cazenovia, and the two activities may be related. Lake Mendota perch undergo a morning migration that is not as pronounced as the evening one (Hasler and Bardach 1949), which may be analogous to the lack of flux observed in Cazenovia at dawn.

Apparent differences between Cazenovia perch and those in other lakes include a longer persistence of schools in Lake Mendota until light levels were near the limits of human vision (Hasler and Villemonte 1953), and a movement from feeding to resting areas in Lake Mendota and in Lake Erie (Scott 1955). In Cazenovia, schools broke up at much higher light levels and the fish remained in the same general area both during the day and night. Emery (1973) found that perch moved continuously but slowly throughout the night and that their coloration at night was paler than during the daytime. In Cazenovia, perch were inactive at night and their coloration was generally darker, with intensified bars and green interspaces. Emery (1978 and personal communication) states that perch in small lakes are less mobile at night than perch in larger lakes and that perch in clearer lakes tend to sleep nearer to structure, even though it is less abundant, than do perch in murkier lakes. Hence activity and behavioral patterns may be influenced by several environmental factors that differ from lake to lake.

Absolute vs. changing illumination as Zeitgebers — Perch generally ceased activities earlier and resumed activities later on overcast days or when the water was turbid. This implies that absolute light levels were important determinants of activity. However, the rate of change of light may have also been an environmental trigger for some activities. For example, PYOY first move showed tighter clustering of data around time relative to sunrise than around actual light levels (see Fig. 2; ratio of coefficients of variation, C.V. = 53:1, light intensity variation > rate of change variation). For activities such as PYOY flux, YOY slowdown, YOY last feed, YOY last group, and YOY speedup, the actual light levels were better correlates of behavior (C.V. ratios = 5.7:1 to 20:1, rate of change C.V. >

light intensity C.V.). For the remaining activities, differences in clustering and C.V. ratios are small (ratios $\leq$4.5:1, 6 of 10 ratios $<$ 2:1). Craig (1977) emphasized the possible importance of an optimum light level as a determinant of activity in *Perca fluviatilis*, while Hasler (1963) suggested that rapidly changing light intensity initiates dusk/dawn migrations in Lake Mendota yellow perch. Apparently either *Zeitgeber* can influence activity, depending on the activity in question and the existing light conditions. W. N. McFarland (Cornell University, Ithaca, N.Y., personal communication) and J. A. Endler (Princeton University, Princeton, N.J., personal communication) have independently suggested that a rate-dependent response might occur where the slope of the light curve is very steep and light is diminishing at a rate similar to or greater than that at which retinomotor movements (= dark adaptation) occur during twilight (see also Ali and Hoar 1959).

Comparisons Within Populations

Ontogenetic differences — In general, YOY perch cease activities earlier in the evening and begin activities later in the morning than their older or larger conspecifics (Table 1). These differences are significant in most cases with respect to both time relative to sunset or sunrise and light levels. The collective nature of the data in the table, representing observations made for different size animals during different twilight periods, underestimates a temporal separation that was obvious in the field. When both YOY and PYOY were observed simultaneously, the differences in timing of activities were dramatic: PYOY were still moving after YOY had stopped in the evening, or PYOY were active before YOY made their first move in the morning.

Other differences between YOY and PYOY relate to the tendency to school and the affinity for structure at night. The increased schooling tendency in YOY was shown both during the daytime and at twilight. YOY seldom occurred apart from schools. These schools stayed together longer than did the schools of larger perch: YOY schools tended to slowdown as a group, whereas larger perch would separate from the school and then slowdown. In the morning, YOY groups formed soon after initial movement, while larger fish were more active before joining other perch and in some cases ignored passing conspecifics rather than swimming with them.

There are at least two explanations for the apparent temporal displacement of the twilight activities of small and large perch. Small fish have small eyes and consequently capture less light; they may therefore have to function at higher light intensities for purely anatomical and physiological reasons. Their small size also makes them more susceptible as prey and they may therefore avoid predation through inactivity during twilight, when light levels fall below some critical value. There is some evidence that the twilight behavior of YOY results from greater predation pressure. The relative timing of group breakup and individual slowdown is reversed from the sequence in larger fish, and YOY remain in schools relatively later. If schooling is an antipredator mechanism, this stronger affinity for schooling may aid in predator avoidance during twilight. In addition, YOY had a greater affinity for structure at night than did most larger perch; this would also afford them shelter from predators such as largemouth bass (*Micropterus salmoides*), smallmouth bass (*M. dolomieui*), rock bass (*Ambloplites rupestris*), and walleye (*Stizostedion v. vitreum*) — fish that are active during twilight or night.

Dawn/dusk comparison — Statistical comparisons indicate some significant differences in the timing of activities at dawn vs. dusk (Table 2). The differences are more striking if, instead of comparing all values

TABLE 1. Comparison of changeover timing, young-of-the-year (YOY) vs. post-young-of-the-year (PYOY) yellow perch, Cazenovia Lake. H_o: no difference in timing of activities; H_a: YOY cease activities earlier in the evening and resume activities later in the morning than PYOY. Probabilities calculated by the Mann–Whitney U Test, one tail (Siegel 1956). * indicates significance at $P \leq .057$; L = light, n = number of observations, P = probability, T = time.

Activity	P_{light}	P_{time}	n_{YOY}	n_{PYOY}
		Dusk		
Flux	$>.05$	$>.05$	1	9*L*, 10*T*
Last feed	.036*	$<.036$*	3	5
Slowdown[a]	$<.05$*	$<.05$*	5	12*L*, 13*T*
Last group	$<.243$	$<.057$*	4	4
Final stop[a]	$<.05$*	$<.10$	3	17*L*, 18*T*
		Dawn		
First move	.086	$<.033$*	4	6
Speedup	.05*	.025*	3	10
First group	.032*	.056*	4	5
First feed	.393	.393	3	5

[a]Different values of n for PYOY slowdown and final stop result from one evening when light measurements were not made.

TABLE 2. Comparison of changeover timing in post-young-of-the-year yellow perch, dusk vs. dawn. H_o: no difference in timing of activities; H_a: activities at dawn occur at lower light levels or earlier times than corresponding activities at dusk. Mann–Whitney U Test, one tail. Abbreviations same as in Table 1.

Activity	P_{light}	P_{time}	n_{dawn}	n_{dusk}
First move vs. last stop	$>.05$	.05*	6	17
First group vs. last group	.056*	.206	4	5
Slowdown vs. speedup	$>.05$	.025*	10	13
First feed vs. last feed	.5	.5	5	5

for an activity, the extreme light and time values in Fig. 1 and 2 are noted. It then appears that PYOY are likely to initiate activity at lower light levels and earlier times in the morning than the corresponding levels at which they cease activity in the evening. Differences in extreme values are also evident for group formation vs. group breakup, slowdown vs. speedup, and for last vs. first feed. These differences may result from light adaptation being more rapid than dark adaptation in some fishes (Ali and Hoar 1959).

ECOLOGICAL PLASTICITY OF PERCH TWILIGHT ACTIVITIES

In making comparisons between populations, I have underplayed the habitat preferences of the perch in different studies. Lake Mendota and Lake Erie perch occurred in large schools over deep water and migrated to shallower water in the evening. The fish observed in Cazenovia were largely restricted to the littoral zone and occurred in small schools or as individuals. Larger schools in the deeper waters of Cazenovia may have migrated into shallower water in the evening. However, tagging studies indicate little mixing between the two regions by either day or night (Helfman 1979). Similarly, Scott (1955) found two subpopulations in Lake Erie. In addition to migrating schools, he observed a group of equal size fish in Rondeau Bay that did not migrate. Hasler and Villemonte (1953) also found nonmigrating schools of perch in deep water in Lake Mendota. It therefore appears that not only can variability in twilight activity be expected between populations, but also within populations. Such subpopulation differences in ecological characteristics have been reported in both yellow and Eurasian perch (Tibbles 1956; Eriksson 1975; Disler and Smirnov 1977; Kelso and Ward 1977; Popova and Sytina 1977). In fact, Il'ina (1973) raised three ecologically different groups — predators, benthophages, and planktivores — from the progeny of one spawning pair of the closely related and perhaps conspecific Eurasian perch.

A high degree of plasticity is therefore characteristic of the yellow perch. Temporal variability may be another aspect of this plasticity. For example, several Cazenovia Lake perch did not engage in all five of the activities of a "typical" twilight sequence. Differences in temporal characteristics between and within populations may result from distinctive genetic variation, or may be a function of relatively fixed responses to environmental conditions that differ between areas. Alternatively, the variability may be part of an overall generalist adaptation to historically varying factors of photic condition, predation pressure, food availability, water clarity, twilight length, and lake size, such that any one of several responses is likely to occur under a given set of conditions. More extensive observation and field manipulation might separate the influence of ecological plasticity from other, less variable, aspects of perch ecology.

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ATP as a Measure of Living Phytoplankton Carbon in Estuaries

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An attempt has been made to evaluate the accuracy of ATP as a measure of living phytoplankton carbon in estuaries. Phytoplankton carbon estimated from ATP was compared to estimates from cell counts. In high biomass samples the agreement between the two estimates was quite good. In the low biomass samples the cell count method underestimated phytoplankton carbon relative to the ATP estimate. This was interpreted to be due in part to low cell counts in these samples. Contamination by microzooplankton (essentially only tintinnids) was, in 14 out of the 18 samples, <3% of the ATP estimated carbon. However, it was as high as 19% in one case. In the lowest biomass samples (<0.5 μg chlorophyll *a*/L) bacterial populations may contribute as much as 50% of the total living carbon. It is concluded that, with caution, ATP is a useful measure of living phytoplankton carbon in estuaries during periods of moderate to high biomass (>1 μg chlorophyll *a*/L for the St. Lawrence). Since carbon to chlorophyll ratios in the high biomass samples varied considerably, a constant ratio appears inapplicable for transforming chlorophyll to carbon.

Key words: ATP, phytoplankton biomass, estuaries, carbon to chlorophyll ratio

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Nous avons tenté d'évaluer la précision de l'ATP comme mesure du carbone du phytoplancton vivant dans les estuaires. Nous avons comparé le carbone du phytoplancton estimé à partir de l'ATP avec des estimations par comptages de cellules. Dans des échantillons à forte biomasse, l'accord entre les deux estimations est assez bon. Dans les échantillons à faible biomasse, les comptages de cellules sous-estiment le carbone du phytoplancton par rapport à l'estimation de l'ATP. Nous croyons que cela est dû en partie au petit nombre de cellules dans ces échantillons. Dans 14 sur 18 échantillons, la contamination par le microzooplancton (essentiellement des tintinnides) est inférieure à 3% du carbone estimé par l'ATP. Dans un cas, cependant, elle s'élevait à 19%. Dans les échantillons de plus faible biomasse (<0,5 μg de chlorophylle *a*/L), les populations bactériennes peuvent contribuer jusqu'à 50% du carbone vivant total. Nous concluons que, si l'on prend certaines précautions, l'ATP est une mesure utile du carbone du phytoplancton vivant dans les estuaires pendant la période où la biomasse varie de modérée à forte (>1 μg de chlorophylle *a*/L pour l'estuaire du Saint-Laurent). Comme les rapports carbone : chlorophylle dans les échantillons de forte biomasse varient grandement, il ne semble pas possible d'appliquer un rapport uniforme qui permettrait de passer de la chlorophylle au carbone.

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THE routine use in field studies of the average growth rate of mixed phytoplankton population (μ, where $dN/dt = \mu N$ and N is a measure of phytoplankton biomass) has been hindered by the lack of a suitable method for estimation of phytoplankton biomass in carbon units. Photosynthesis or production (dN/dt) is frequently estimated using the ^{14}C method (Strickland and Parsons 1972) but biomass (N) is routinely estimated by chlorophyll *a*. Photosynthesis at saturating light intensity normalized by biomass in chlorophyll *a* units (sometimes defined as assimilation ratio or number) has been used in distributional studies as a measure of physiological state, or as a measure of species composition differences. If cellular carbon/chlorophyll *a* ratios are assumed to be relatively constant, growth rate, and by inference physiological state, can be estimated from assimilation numbers (see Walsh et al.

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1974; Malone 1977). However, due to the highly variable carbon/chlorophyll *a* ratio with growth phase and species composition (range of 20–200 reported by Eppley 1972), a change in assimilation ratio does not necessarily reflect a change in growth rate; conversely, a constant assimilation ratio could be measured during variable growth phases or physiological states. In addition to permitting a routine measure of growth rate and carbon/chlorophyll *a* ratio, an accurate method for the estimation of living phytoplankton carbon would permit direct coupling of phytoplankton dynamics with higher trophic levels which are routinely studied in carbon units (zooplankton biomass, grazing rate, etc.).

Several indirect methods for estimating living phytoplankton carbon in natural waters in addition to the above mentioned use of chlorophyll *a* have been used. Steele and Baird (1961) and Platt and Conover (1971) have estimated the cellular carbon/chlorophyll *a* ratio from the regression of particulate carbon (which includes living and nonliving fractions) on chlorophyll *a*. This assumes, perhaps unrealistically, that the cellular ratio in the diverse samples used for the regression has a constant value, and that detrital carbon levels are relatively constant and thus independent of the phytoplankton carbon. The potential errors in the regression method were discussed in detail by Banse (1977). Reid et al. (1970) estimated cellular carbon from cell numbers and cell volumes and a subsequent transformation to carbon units using either the regression of Mullin et al. (1966) or Strathman (1967), which we will describe in this study as the "cell volume transformation" method. Although this approach yields additional high quality data on species composition, it is too laborious for routine analyses and is of low precision due to accumulative errors from cell counting difficulties (Jassby 1975). Also, variation in the ratio of cell volume to cell carbon with the nutritional status of the cell (Caperon and Meyer 1972; Eppley and Renger 1974) is not considered in the above regressions. Finally, Holm-Hansen (1970, 1973) has suggested the use of ATP as a measure of living phytoplankton carbon since the carbon/ATP ratio was observed to be constant and independent of species and physiological state. Sakshaug (1977) observed an order of magnitude range in this ratio with changes in nutrient availability and related growth rates. He mentioned, however, that the constant ratio suggested by Holm-Hansen is appropriate when phosphate is not limiting and growth rates are near maximal. Particulate ATP is restricted to living cells but includes an unknown level of "contamination" from heterotrophs (bacteria and microzooplankton), even if selective prefiltration of water samples is affected in order to remove the larger zooplankton. However, in the euphotic zone of eutrophic waters it is reasonable to expect that phytoplankton cells are the predominant source of particulate ATP. The observation of Eppley et al. (1977) that nonphytoplankton carbon accounts for approximately 10% of the living carbon (prefiltered through 200-μm mesh) in the California Current, and the limited observations of close agreement between field growth rates estimated using biomass in carbon units, derived respectively from "cell volume transformation" and ATP (Eppley 1972), support the above supposition concerning negligible contamination of particulate ATP by heterotrophs in the euphotic zone. Banse (1977), however, reported that zooplankton may contribute more than 10% of the particulate ATP. If the carbon/ATP ratio of the zooplankton contribution is greater than that of the phytoplankton (Skjoldal and Bamstedt 1976), heterotrophic contamination may be considerable.

In this study, a comparison is made between living phytoplankton carbon estimated by two methods, ATP and cell volume transformation, on a subset of samples taken during a study of temporal phytoplankton distributions in the lower St. Lawrence Estuary, and an evaluation of the heterotrophic contribution to particulate ATP measures is made. The distribution of the ATP/chlorophyll *a* ratio is also considered. In this estuary, in which nutrient limitation is minimal (Steven 1974; Coote and Hiltz 1975; Sinclair et al. 1976), the assumption of a constant carbon/ATP ratio is probably justifiable and the regressions of cell volume on cell carbon by Strathman (1967) and Mullin et al. (1966) are probably appropriate. But contamination of the particulate ATP by heterotrophs associated with estuarine detritus may be of more importance than in an offshore environment such as the California Current. It was hoped that this comparison, and the estimation of heterotrophic contamination, would aid in the evaluation of ATP as a measure of living phytoplankton carbon in estuarine studies, as it has been cautiously used in the St. Lawrence Estuary (Sinclair 1978) and in Narragansett Bay (Durbin et al. 1975).

Methods

The sampling procedures and the station position occupied during a survey of the lower St. Lawrence Estuary and during a temporal study at a single station are described in Sinclair (1978). Each week triplicate subsamples for chlorophyll *a* and particulate ATP and duplicate subsamples for phytoplankton cell counts were taken from 12-L Niskin bottles at each of six depths. For ATP analyses, the 150-mL subsamples were filtered through 0.8-μm, 22-mm diam Millipore filters, the filters boiled in tris buffer and frozen. For chlorophyll *a* analyses, 250-mL subsamples were filtered through 25-mm diam Whatman GF/C filters and frozen in a desiccator. Samples for cell counts were preserved with neutralized formalin (1%). The ATP samples were prefiltered through 404-μm Nitex netting to remove larger zooplankton.

Chlorophyll *a* was determined by the modified method of Yentsch and Menzel given in Strickland and Parsons (1972). The fluormeter was calibrated regularly with pure chlorophyll *a* Sigma Chemical Co. The particulate ATP samples were assayed by the luciferin–luciferase method of

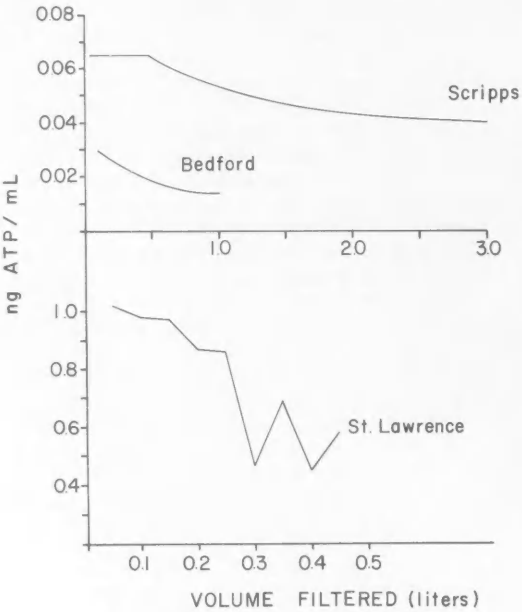

FIG. 1. Effect of the sample volume filtered on the accuracy of the ATP method. Data from Scripps and Bedford were taken from Sutcliffe et al. 1976.

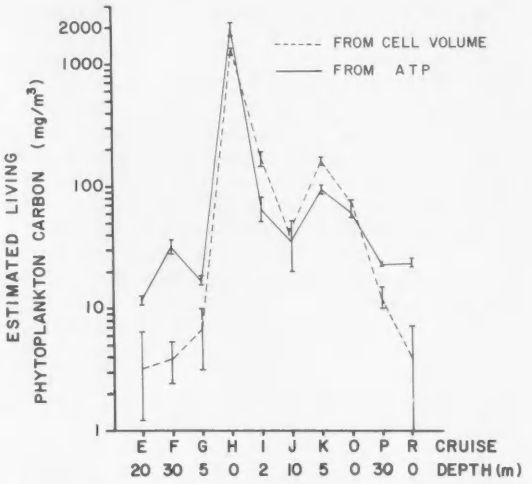

FIG. 2. Two independent estimates of living phytoplankton carbon measured on samples from various depths collected at the Rimouski station during a weekly sampling program (June–October 1974).

Holm-Hansen and Booth, as described in Strickland and Parsons (1972). Sutcliffe et al. (1976) indicate that the linear relationship between volume of the sample filtered and the resultant concentration of ATP estimated is valid only up to a critical volume, and that this critical volume varies with the environment sampled. A test of this relationship was carried out and the results (Fig. 1) indicate that the critical volume at this date was ~250 mL. Thus the volume chosen for routine sampling, 150 mL, was probably suitable throughout the sampling program, being considerably lower than the critical value found for the St. Lawrence Estuary, as well as that reported for Bedford Basin and La Jolla Bay. ATP values were converted to living phytoplankton carbon estimates by multiplying by 250 (Holm-Hansen 1970; 1973; Sakshaug 1977).

From the total sample collection (20 cruises, 6 depths) 10 date-depth conditions were chosen, in a stratified-random fashion, to represent the observed range in species composition, phytoplankton biomass, and depth. For each of the 10 conditions chosen the duplicate preserved samples were treated to provide an independent estimate of living

TABLE 1. Comparison of living phytoplankton carbon estimated from ATP concentration and from cell volume transformation.

	Method A			Method B			
Sample	ATP estimated carbon (μg/L)	Coef. of variation ($s/x \times 100$)	Carbon/chlor ratio	Cell volume estimated carbon (μg/L)	Coef. of variation ($s/x \times 100$)	Carbon/chlor ratio	Method A / Method B
E (20 m)	11.72±0.48	4.1	83.7	3.31±2.88	87.0	23.6	3.5
F (30 m)	32.22±3.60	11.2	107.4	3.87±1.38	35.7	12.9	8.3
G (5 m)	17.07±1.48	8.7	77.6	6.62±3.43	51.8	30.1	2.6
H (surface)	1983.8±224.7	11.3	87.8	1317.97±8.18	0.62	59.7	1.5
I (2 m)	65.81±16.04	24.4	33.6	114.35±5.09	4.5	58.3	0.6
J (10 m)	35.88±16.31	45.5	48.5	41.87±3.30	7.9	56.6	0.9
K (5 m)	95.14±6.75	7.1	53.8	164.38±5.20	3.2	92.9	0.6
O (surface)	61.68±7.03	11.4	74.3	72.35±3.40	4.7	87.2	0.9
P (30 m)	22.76±0.15	0.66	51.7	12.16±2.92	24.0	27.6	1.9
R (surface)	23.62±1.69	7.2	84.4	4.16±2.81	67.5	14.8	5.7

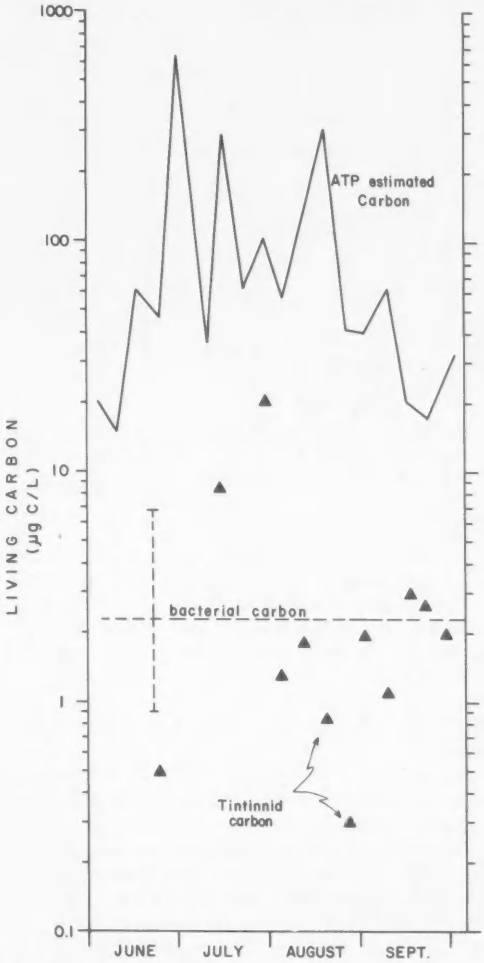

FIG. 3. ATP estimated carbon and heterotrophic contamination at 2 m depth at the Rimouski station during a weekly sampling program (June–October 1974).

phytoplankton carbon from the cell counts. Species composition, cell counts, and cell dimensions were accomplished by the Utermöhl method (Lund et al. 1958) with an inverted phase contrast microscope (Wild M40). To provide an error estimate for the final biomass values, triplicate aliquots of equal volume from each of the two subsamples were analyzed. The volume of the aliquots (1, 10, or 100 mL) depended on the cell concentrations in the sample. The mean abundances for each species were converted to cell volumes following the criteria established by Kovala and Larrance (1966). Depending on the general morphology of each species appropriate dimensions for volume estimation were measured (e.g. thickness and diameter for *Coscinodiscus* spp., which are disc-shaped). Volumes were subsequently transformed to carbon using the regressions reported by Mullin et al. (1966) and Strathman (1967). The accumulated errors associated with the biomass estimates were calculated following Venrick (1971). Weekly microzooplankton abundances at 2 m depth were estimated using the Utermöhl method (100-mL aliquots were counted).

Results and Discussion

The 10 estimates and their associated errors of phytoplankton biomass from ATP and cell volume transformation are shown in Table 1 and Fig. 2. In the high biomass samples, greater than about 10 μg C/L by cell volume transformation method (H, I, J, K, O), the agreement between the two estimates is good considering the low precision of the methodology. In the low biomass samples (E, F, G, P, R), whether they are surface or near bottom samples (station depth ~35 m), the cell volume transformation method seriously underestimated carbon relative to the ATP method. From inspection of the respective carbon/chlorophyll *a* ratios, e.g. 107 and 13 for sample F (30 m) and 84 and 15 for R (surface), the differences are in part due to low cell volume transformation estimates. The low carbon estimates from cell counts in these samples could be due to destruction of fragile phytoplankton species by formalin or to insufficient volume of sample counted relative to cell concentrations. Alternatively, the cell volume transformation estimate may be an accurate estimate of phytoplankton carbon, and the ATP estimates high due to contamination by animal and bacterial ATP.

The importance of contamination by heterotrophs has been estimated by indirect methods and is shown in Fig. 3. Tintinnid abundances (essentially the only microzooplankton group observed, Sinclair 1978) observed each week were multiplied by an average factor (0.0042 μg C/tintinnid), derived from table 3 of Beers et al. (1975), to provide a microzooplankton biomass value in carbon units. Clearly, the tintinnid species composition in the North Pacific central gyre is different from that in a subartic estuary, but its carbon biomass–population abundance relationships are derived from an average of six stations over a broad depth range and thus should be representative of a diverse species assemblage. In 14 out of the 18 samples considered, estimated microzooplankton contamination was $<3\%$ of ATP-derived carbon but in one sample it was as high at 19%. The bacterial biomass for several stations in the Baltic Sea has been estimated by Dawson and Gocke (1978), and since this is a relatively similar hydrographic site to our sampling region it may serve to suggest an approximate magnitude of bacterial biomass. The average surface layer (0–50 m) value observed, and the range (0.9–6.4 μg C/L), are indicated in Fig. 3. A second indirect estimate can be made by multiplying observed bacterial population abundances in estuaries by the range in values observed for carbon content per cell ($1.7–8.3 \times 10^{-7}$ μg C/cell, Hamilton and Holm-Hansen 1967). In this way, 10^6 cells/L

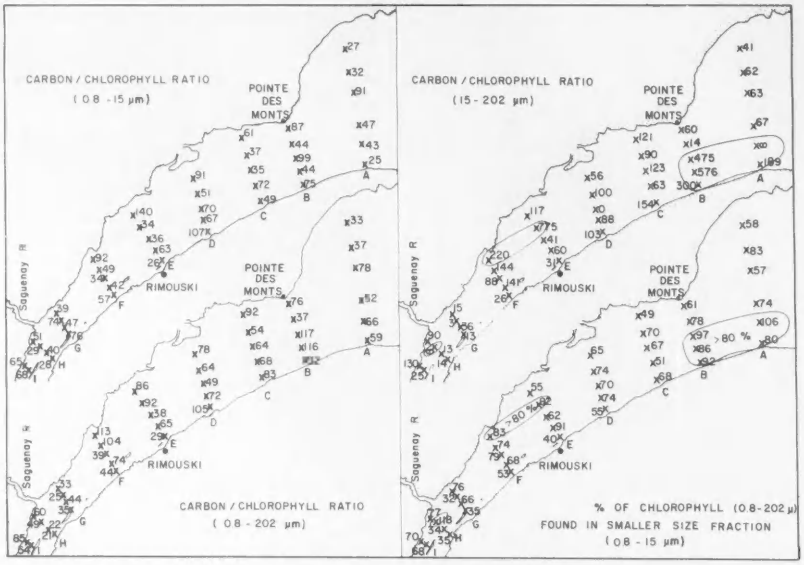

FIG. 4. Spatial distribution of ATP-derived carbon/chlorophyll ratio of three size fractions measured at 5 m depth within the lower St. Lawrence Estuary during July 1975. The percentage of the total chlorophyll (0.8–202 μm) which is found in the small size fraction (0.8–15 μm) is indicated on the lower right map. Stations at which nannoplankton chlorophyll was >80% of the total chlorophyll are circled in right half.

would represent 0.2–0.8 μg C/L or <10% of the lowest carbon value in Fig. 3. Clearly the extent of bacterial contamination cannot be defined without direct estimate of bacterial biomass in the lower St. Lawrence, but from the above considerations bacteria should only be an important contribution to particulate ATP in samples of very low phytoplankton biomass. This conclusion agrees with the theoretical treatment by Jassby (1975).

Spatial distribution of the ATP-derived carbon to chlorophyll *a* ratios from samples at 5 m depth within the lower St. Lawrence Estuary (Fig. 4) allows some inferences to be drawn on the degree of heterotrophic contamination of the ATP samples. For the 0.8–202-μm and the 0.8–15-μm size categories reasonable values with no clear geographical pattern were observed with total ranges of, respectively, 21–117 and 25–140. In contrast, unreasonable ratios both too high and too low are obtained for the 15–202-μm size category. To a certain degree, the high ratios are associated with the patches dominated by nannoplankton (circled stations in right half of Fig. 4). The low values (0, 3, 13, 4) indicate that the combined errors in the methodology significantly affect the ratio of the larger size category. Within this size-class (15–202 μm) the concentrations of carbon and chlorophyll *a* can be minimal, especially in the nannoplankton–dominated samples which are in general associated with low biomass. Thus, small analytical errors in the chlorophyll *a* or carbon estimates can lead to either very high or low ratios. In addition to the analytical error, certain of the high values observed for the ratio of the 15–202-μm size fraction are no doubt due to microzooplankton contamination. The contamination may not be too serious in that the correlation coefficients between carbon and chlorophyll *a* for the two categories, 0.8–15 μm and 0.8–202 μm, are similar (0.69 and 0.66, respectively). One would expect a reduced correlation between carbon and chlorophyll *a* in the broader size-class (0.8–202 μm) relative to the nannoplankton if zooplankton contamination was important.

The accuracy of ATP as a measure of living phytoplankton carbon in general is debatable, but in estuaries in which nutrient limitation is often negligible (and thus the constant carbon/ATP ratio suggested by Holm-Hansen may be appropriate) it is a moderately accurate estimate when the phytoplankton biomass is medium to high (i.e. >1 μg chlorophyll *a*/L). In the lower St. Lawrence Estuary, unless exceptionally high bacterial biomasses are present, contamination by heterotrophs does not appear to be large. Nevertheless, phytoplankton carbon estimated by ATP in the lower St. Lawrence and other estuaries should be interpreted with caution. For a particular estuary, nutrient availability and potential heterotrophic contamination should be considered before (or concurrent with) measures of particulate ATP. Nutrient-limited growth conditions will lead to an underestimate of phytoplankton carbon from ATP,

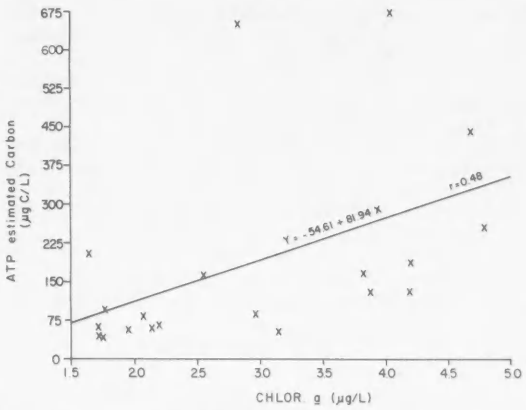

FIG. 5. ATP estimated carbon and chlorophyll concentrations observed in the high biomass samples at the Rimouski station during a weekly sampling program (June–October 1974).

whereas heterotrophic contamination will lead to an overestimate of phytoplankton carbon from ATP.

Considerable variability with depth and from week to week was observed in the estimated phytoplankton carbon/chlorophyll *a* ratio, especially during periods of relatively high biomass when the ATP-derived phytoplankton carbon is reliable. In Fig. 5 the higher biomass samples illustrate the variability in the carbon/chlorophyll *a* ratio and the relatively poor correlation between carbon and chlorophyll *a* ($r = 0.48$, which is nonsignificant at the 1% confidence level). Since a wide range of values of the ratio have been observed in cultures (Eppley 1972; Sakshaug 1977), such variability in the ratio is not unexpected in an high energy environment like the lower St. Lawrence, in which different species assemblages of variable physiological state are observed from week to week (Sinclair 1978). The variable ratio does sugggest, however, the inapplicability of using a constant ratio for estimating carbon from chlorophyll *a*, or of using regression analysis of chlorophyll on particulate carbon for an estimate of the carbon/chlorophyll *a* ratio, in this environment. The spatial variability in the ratio within the lower estuary (Fig. 4) supports this observation.

We conclude that with sufficient caution (e.g. consideration of phytoplankton nutrient status and level of heterotrophic contamination), particulate ATP is a useful measure of living phytoplankton carbon in estuaries.

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Summer Plankton Distributions Associated with the Physical and Nutrient Properties of the Northwestern Gulf of St. Lawrence

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Two transects, north–south and east–west, through the Anticosti gyre and the Gaspé current were sampled for physical properties, nutrient and plankton concentrations over a 5-d period during July 1976. Temperature, salinity, σ_t and nutrient isolines formed a dome characteristic of cyclonic circulation with the apex within the Anticosti gyre, but a strong shallow thermocline prevented nutrient enrichment of the surface mixed layer. Surface nitrate and silicate concentrations were very low, except for two anomalies, throughout the region sampled, with phosphate concentrations higher. Phytoplankton biomass was uniformly low and dominated by nannoplankton ($<15\ \mu m$) at all stations except that in the Gaspé current at which netplankton (15–202 μm) was important and the total biomass considerably higher. The higher biomass and importance of larger celled species suggest higher nutrient turnover in the Gaspé current. *Nitzschia delicatissima* dominated the species composition in the Gaspé current, dinoflagellates and microflagellates being of more importance within the gyre and along the north shore. Zooplankton biomass was distributed fairly uniformly over the entire region but species composition at the shallower stations was characterized by small copepod species (*Pseudocalanus* sp., *Acartia* sp.) and fish larvae, the gyre stations being characterized by larger copepod species (*Calanus* sp.), euphausids, and decapod larvae. Physical properties, nutrients, and plankton were distributed quite uniformly except for the marked variability in the measurements made in the strongly advective Gaspé current station. Distributions of derived parameters that were estimated at 5 m depth suggest nutrient limitation at most stations within the gyre, with the exception of the two stations at which surface nutrient anomalies were detected, while at the nearshore stations, in the Gaspé current and along the north shore, better physiological state and lack of nutrient limitation were observed. Short-term variability in these parameters complicate geographic interpretation, but trends were discernable. Estimated daily primary production was an order of magnitude less than that reported previously.

Key words: Anticosti gyre, Gaspé current, primary production, nutrient limited growth

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Sur une période de cinq jours en juillet 1976, nous avons effectué deux coupes, nord-sud et est-ouest, dans le tourbillon d'Anticosti et le courant de Gaspé en vue d'en déterminer les propriétés physiques et les concentrations d'éléments nutritifs et de plancton. Les lignes d'égale échelle de température, salinité, et éléments nutritifs forment un dôme caractéristique d'une circulation cyclonique, avec sommet à l'intérieur du tourbillon d'Anticosti, mais une thermocline prononcée à faible profondeur empêche l'enrichissement en éléments nutritifs de la couche mélangée superficielle. Les concentrations de nitrates et de silicates à

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la surface sont très faibles, sauf pour deux anomalies, dans toute la région échantillonnée, avec concentrations plus fortes de phosphates. La biomasse phytoplanctonique est uniformément faible et dominée par le nannoplancton (<15 μm) à toutes les stations, sauf celle du courant de Gaspé à laquelle le plancton tamisé (15 à 202 μm) était important et où la biomasse totale était beaucoup plus forte. La biomasse plus forte et l'importance d'espèces à plus grosses cellules donnent à penser qu'il y a un meilleur renouvellement des éléments nutritifs dans le courant de Gaspé. *Nitschia delicatissima* est l'espèce dominante dans le courant de Gaspé; alors que dans le tourbillon et le long de la côte nord, les dinoflagellés et les microflagellés sont plus importants. La biomasse zooplanctonique est répartie assez uniformément dans toute la région, mais la composition spécifique aux stations moins profondes est caractérisée par des petites espèces de copépodes (*Pseudocalanus* sp., *Acartia* sp.) et des larves de poissons. Les stations du tourbillon sont caractérisées par des espèces de copépodes plus grosses (*Calanus* sp.), des euphausides et des larves de décapodes. Les propriétés physiques, les éléments nutritifs et le plancton sont répartis assez uniformément, sauf pour une variabilité marquée des mesures effectuées à la station du courant de Gaspé, fortement advectif. La distribution des paramètres dérivés estimés à une profondeur de 5 m suggère une limitation des éléments nutritifs à la plupart des stations dans le tourbillon, à l'exception de deux stations auxquelles nous avons décelé des anomalies de surface dans la répartition des éléments nutritifs. Par ailleurs, aux stations près de la côte dans le courant de Gaspé et le long de la côte nord, nous observons une meilleure condition physiologique et l'absence de limitation des éléments nutritifs. La variabilité à court terme observée dans ces paramètres complique l'interprétation géographique, mais on peut discerner des tendances. La production primaire quotidienne estimée est moindre d'un ordre de grandeur que celle qu'on avait mentionnée auparavant.

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THERE are several indications that the northwestern Gulf of St. Lawrence, specifically the region between the lower estuary and Anticosti Island, is highly productive. The major features of the circulation, the relatively permanent Anticosti gyre (El-Sabh 1976) and the Gaspé current, are shown in Fig. 1. Steven (1974) reported continued extremely high levels of primary production throughout this region from April to October. Platt (1972) observed high levels of phytoplankton biomass within the Gaspé current and Sameoto (1976) linked high euphausid population abundances with the phytoplankton stock. Jacquez et al. (1977) and Bailey et al. (1977) reported high capelin larvae and juvenile abundances in the Anticosti gyre during the autumn and presumably winter months. Also, the center of a shrimp fishery (*Pandalus borealis*) is located within the gyre (Couture 1971) and high yields of both pelagic and groundfish species are taken from the Gaspé current (S. Messieh, St. Andrews laboratory, personal communication). The nutrient transport mechanism, however, supporting the reported high food chain and an active local fishery are poorly understood. Steven (1974) suggested that nutrient transport via the estuary accounts for the observed productivity over the broad area of interest. He proposed the existence of a nutrient pump at the mouth of the Saguenay, due to the high amplitude internal waves that are generated in this area. Sinclair et al. (1976) estimated that estuarine transport could not account for the high levels of primary production reported in this region by Steven, but that if the major portion of the transport was concentrated along the south shore, estuarine transport could influence the production within the Gaspé current (note that the estimates for nitrogen nutrient transport in Sinclair et al. (1976) have been corrected upwards by 14).

It was suggested that local sources of nutrient supply within this region of high production should be considered. For instance, entrainment within the Gaspé current which has two layer estuarine circulation features, in addition to the advective transport from the estuary, may contribute nutrients to the surface layer. Also, El-Sabh (1976) discussed the potential importance of upwelling within the cyclonic Anticosti gyre as a nutrient transport mechanism. Legendre and Watt (1970), Nival (1976), and Sargent and Walker (1948) have reported the importance of upwelling on phytoplankton production within cyclonic gyres. The Anticosti gyre has been identified from geostrophic calculations but the associated hydrological and plankton distributions have not been described, although the "doming" of nutrient isolines has been observed in this region (Coote and Hiltz 1975).

The aim of this study is to describe the oceanographic features, and their short-term variability (hours to several days), associated with the circulation pattern in the region of interest. It was thought that if upwelling within the gyre and/or entrainment within the Gaspé current were significant processes, their impact would be identifiable during a period of potential nutrient limitation, the midsummer months. An active nutrient source would be observable from nutrient distributions, phytoplankton species composition, and perhaps the average physiological state of the component popu-

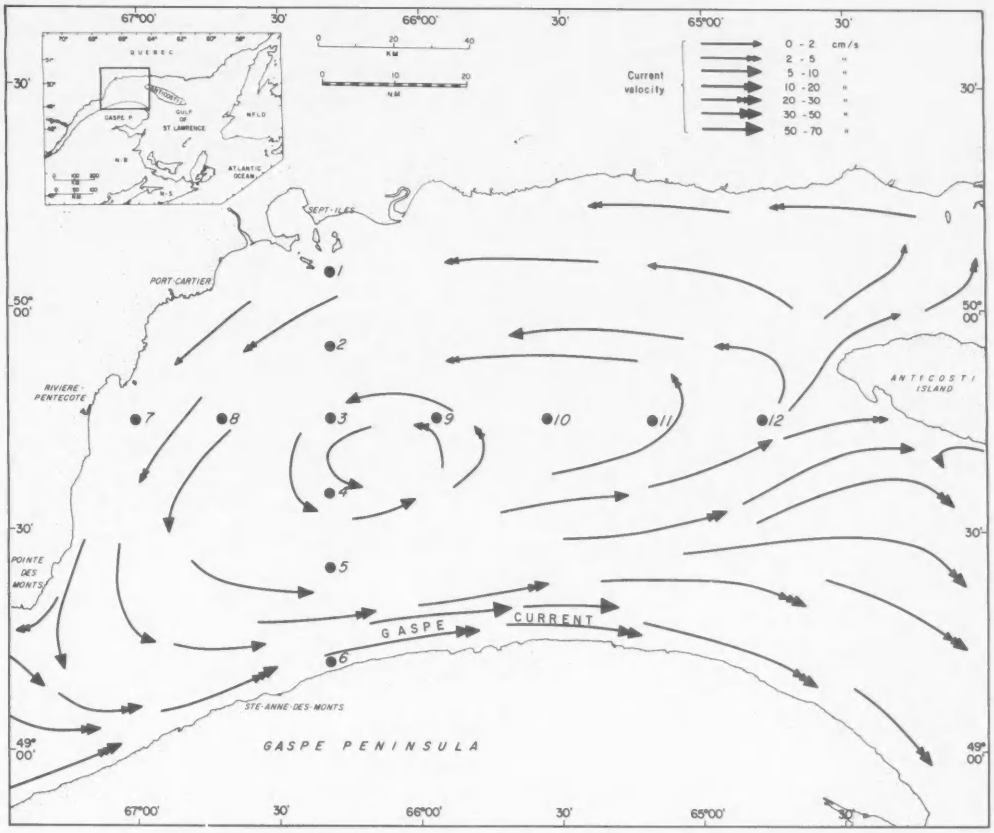


FIG. 1. Surface circulation (from El-Sabh 1976) and station positions in the study area.

lations. With this rationale in mind the northwestern Gulf was investigated during mid-July.

Materials and Methods

The stations occupied during the cruise on the C.S.S. *Dawson* (July 19–25, 1976) are indicated in Fig. 1. The north–south transect (stations 1–6) was occupied twice, at the beginning and at the end of the cruise. Stations 1, 3, and 6 were each occupied for 13 h, with sampling every hour for the physical parameters and every 2 h for the chemical and biological parameters. At each station, after STD profiling and Secchi disk reading, a hydrocast with 12-L Niskin bottles at 0, 5, 15, 35, 65, 115, and 265 m (depth permitting) was taken. Subsequently a single vertical haul with a 75-cm net (202-μm mesh) equipped with a TSK flow meter was taken from near bottom to the surface for zooplankton biomass and species composition. The samples were preserved in 4% formalin neutralized with sodium borate. A 400-mL subsample from the 5-m depth Niskin was preserved in 1% neutralized formalin for phytoplankton and microzooplankton counts. From each Niskin 100-mL subsamples to which several drops of chloroform were added were drawn for nutrient analyses. Nitrate, phosphate, and silicate were analyzed on board within several hours with a Technicon Auto-analyser. For chlorophyll *a* and ATP analyses, subsamples of 250 mL from each Niskin were prefiltered through 202- and 15-μm Nitex mesh. The subsampling procedure from the 5-m depth Niskin was more elaborate since chlorophyll and ATP for the two size categories (0.8–15 μm and 0.8–202 μm) were each subsampled in triplicate and ^{14}C subsamples (also in triplicate) drawn for deck incubation. Three 300-mL subsamples for photosynthesis estimation were drawn into BOD bottles, innoculated with 10 μCi of sodium bicarbonate ($NaH^{14}CO_3$), and incubated at saturating light intensity at near surface temperature for 5 h. Periodically controls were taken for nonbiological absorption of ^{14}C filters by immediately filtering samples subsequent to their innoculation (Williams et al. 1972). After the 5-h incubation half of each sample was passed through 202-μm mesh and the other half through 15-μm mesh. The divided samples were then filtered through 0.8-μm Millipore filters and placed in 10 mL of Aquasol. We decided to "postfilter"

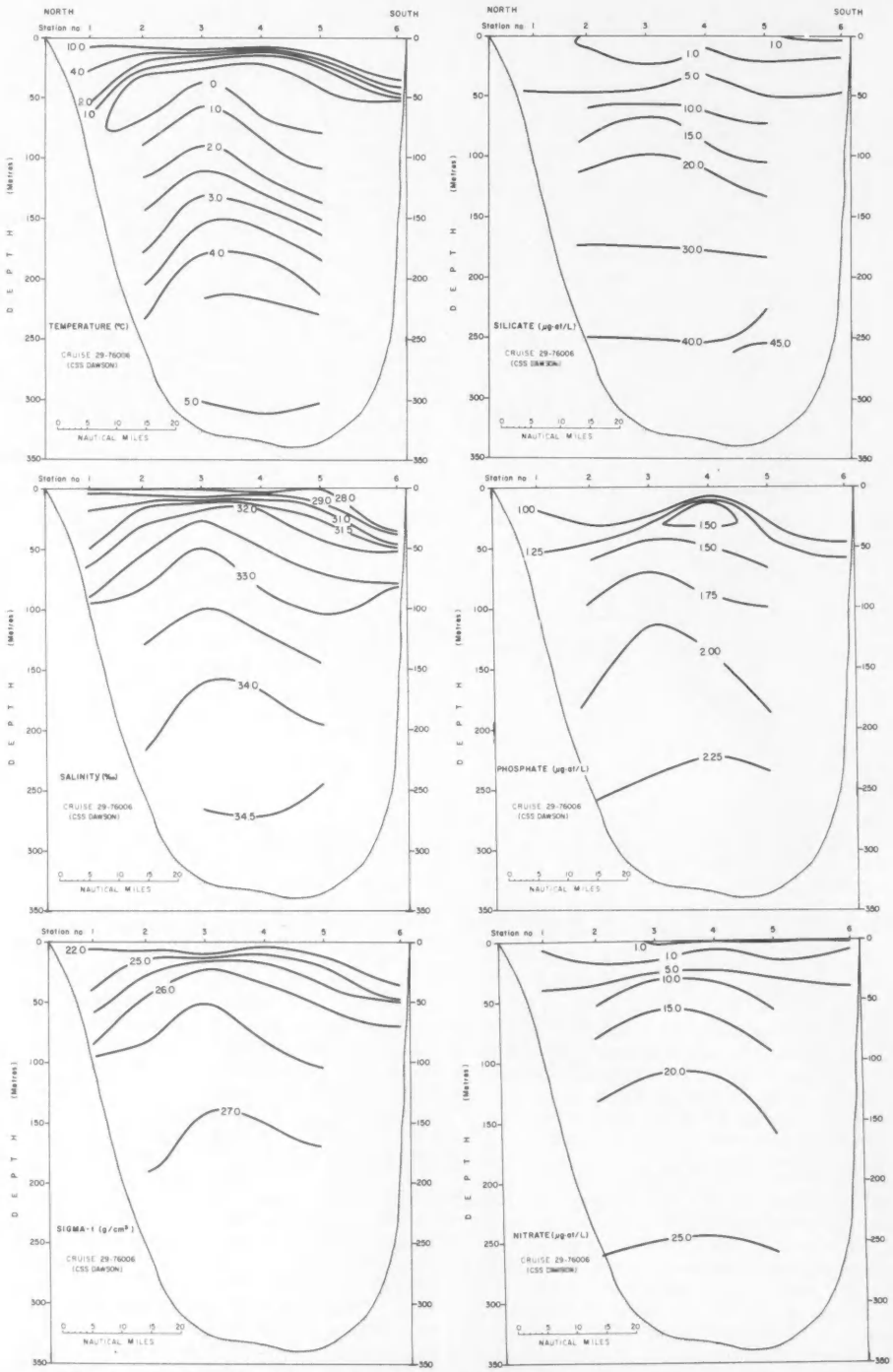


FIG. 2. Isolines of T, S‰, σ_t, and nutrients across the north–south transect, station 1–6.

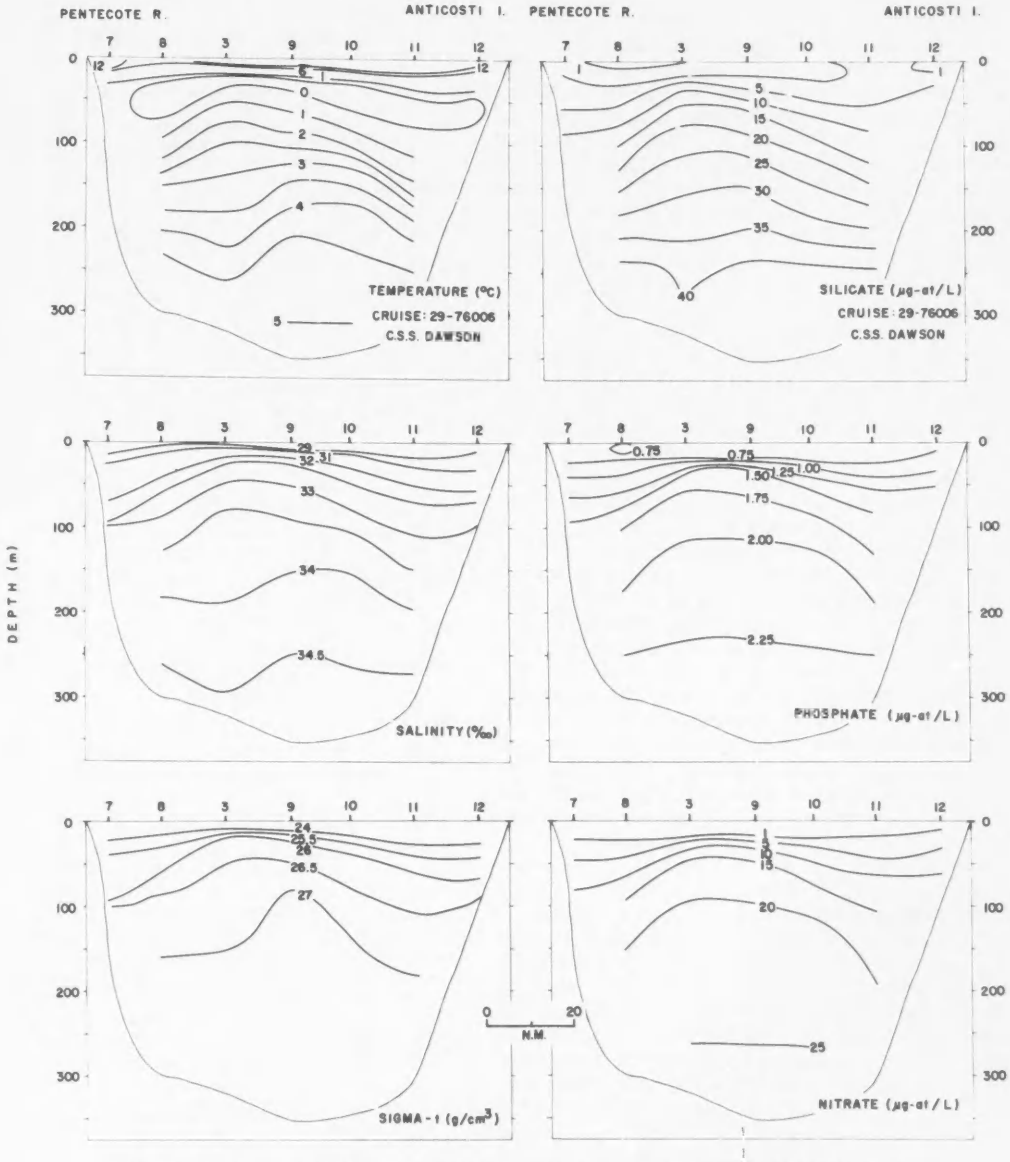


FIG. 3. Isolines of T, S‰, σ_t, and nutrients across the east–west transect, stations 7–12.

the ^{14}C samples to avoid the possible artificial effect of breaking long chains during prefiltration (McCarthy et al. 1974).

Chlorophyll *a*, ATP, and carbon assimilation were determined following Strickland and Parsons (1972). Phytoplankton living carbon was roughly estimated by multiplying the ATP values by 250 (Holm-Hansen 1970, 1973). The carbon to ATP ratio has been shown to increase to as high as 2500 under severe nutrient limitation (Sakshaug 1977), but in that study it was suggested that the constant value of Holm-Hansen is appropriate if phosphate is not limiting, which was found to be the case in this study. From microscopic observations of the 5-m depth samples heterotrophic contamination was minimal with respect to the phytoplankton carbon contribution. Nevertheless, the tenuous accuracy of living phytoplankton carbon estimates

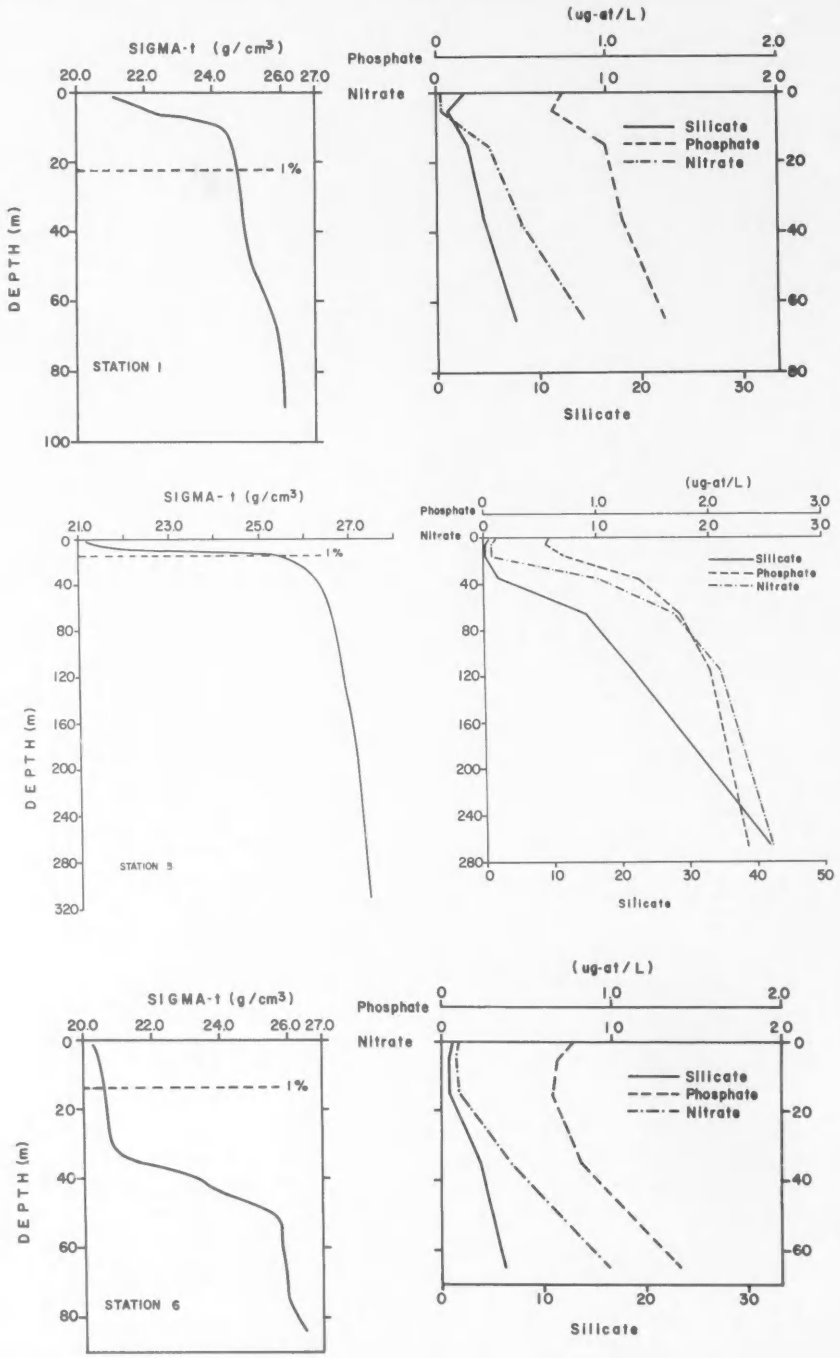


FIG. 4. Depth profiles of σ_t, and nutrients at stations 1, 3, and 6, with the approximate depth of the euphotic zone indicated.

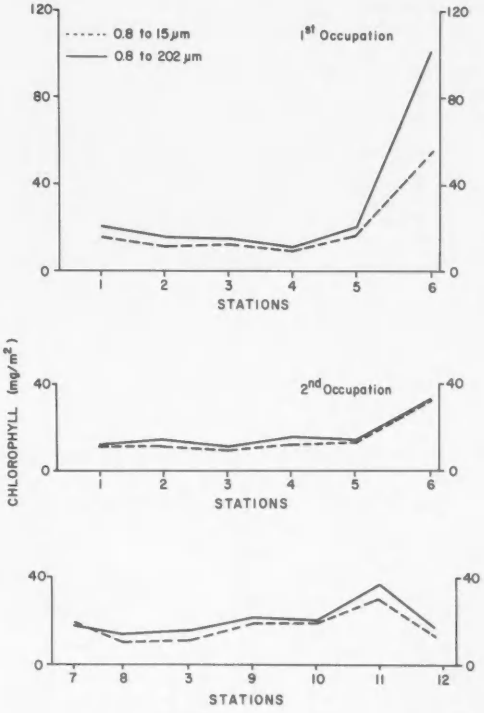


FIG. 5. Water column chlorophyll (in two size categories) concentration observed across the north–south (two occupations) and east–west transects.

from ATP is recognized and thus must be interpreted with caution. The average specific growth rate (μ) at 5 m was calculated from the carbon assimilation and ATP-derived carbon estimates following the approach of MacAllister et al. (1964) and has the units h^{-1}. The derived parameters, growth rate, and light-saturated carbon assimilation divided by chlorophyll *a* are termed production/biomass ratios (P/B) in this study; and the carbon to chlorophyll *a* ratio abbreviated by C/chlor.

Phytoplankton composition was estimated using the Utermöhl technique (Lund et al. 1958) with an inverted phase contrast microscope (Wild M40).

Results and Discussion

The isolines of S‰, temperature, σ_t, and the inorganic nutrients for the two transects, north–south and east–west, are shown in Fig. 2 and 3. Figure 2 is derived from the first occupation of this transect, the distributions being essentially identical to those observed during the second occupation a few days later. The "doming" of the isolines within the gyre for each of the parameters, which is characteristic of cyclonic gyres, is well developed. For example, the midwater temperature minimum (<0°C water) is displaced by about 25 m. The amplitude of isoline displacement increases somewhat with depth; the 27.00 σ_t isoline, for example, covers about 50 m (Fig. 2 and 3). The upward movement of the central water mass results in a shallower surface mixed layer in the center of the gyre than in the surrounding water but does not result in high nutrient concentration in the surface layer. The deep mixed layer of lower salinity within the Gaspé current (station 6, Fig. 2) is to be noted. The nutrient isolines come closer to the surface at station 4 and an anomalous surface distributions are observed at stations 8 and 11 (Fig. 2 and 3, respectively). The activity of the phytoplankton populations at these two stations is discussed below.

The vertical profiles for the same parameters at three of the stations (1, 3, and 6) illustrate the difference in the nature of density stratification across the north–south transect in relation to the approximate depth of the euphotic zone (depth of 1% light penetration) and surface layer nutrient depletion (Fig. 4). At stations 1 and 3 where the euphotic zone is deeper than the mixed layer, silicate and nitrate concentrations are depleted within the upper rather shallow mixed layer, with phosphate levels being relatively higher. Within the Gaspé current, however, at station 6 nutrients increase above the pycnocline, but below the euphotic zone. Due to the shallow mixed layer relative to the surface water turbidity at stations 1 and 3, the 1% light level being close to the base of the pycnocline, one might expect active growth of phytoplankton at this level where the nutrient availability is higher. At station 6, where the mixed layer is considerably deeper than the euphotic zone, it is of interest to note that the critical depth (Sverdrup 1953) is approximately 100 m during July. Thus, providing that there is a nutrient source during the summer months, through advection or entrainment, active mixed layer phytoplankton growth is to be expected.

The distribution of depth-integrated phytoplankton biomass for the two size categories considered, which was estimated both from the chlorophyll *a* and ATP measures, indicates the same general features; and for economy of space only the chlorophyll *a* results are shown (Fig. 5). The Gaspé current, as represented by station 6, is considerably more productive than the Anticosti gyre and the north shore stations and is the only region characterized by a large proportion of netplankton (15–202 μm). There is considerable short-term variability within the Gaspé current since in the second occupation, a couple of days later, the depth-integrated chlorophyll *a* decreased by 50% and the presence of large-celled phytoplankton species diminished. This effect is no doubt due to sampling in a strongly advective environment, the current speeds being about 18–36 km/d (Trites 1971; El-Sabh 1976) rather than due to short-term temporal phenomena within a parcel of water. The only other features are the relative constancy in the biomass and dominance by nannoplankton at the other stations, both geographically and

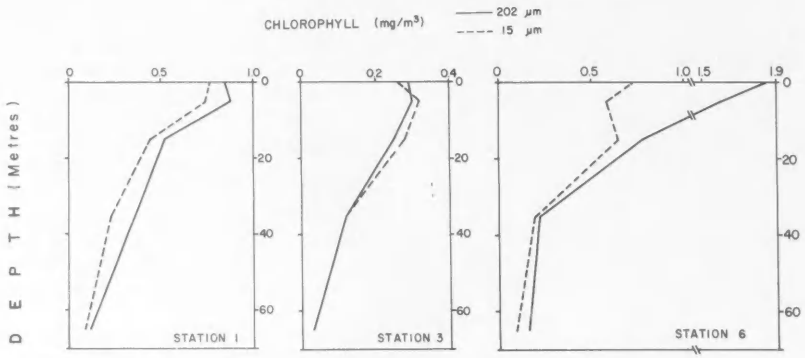


FIG. 6. Depth profile of chlorophyll (in two size categories) at stations 1, 3, and 6.

temporally (stations 1–5 over a 2-d period), and the higher phytoplankton stock at station 11. From inspection of Fig. 3, higher surface layer silicate values were previously noted at station 11; but no corresponding features were observed in the other hydrological parameters.

The vertical profiles of chlorophyll concentration for the same three stations (Fig. 6) indicate a fairly uniform decline with depth, and at station 6 a parallel decrease in the relative importance of "netplankton." Increased phytoplankton biomass at the pynocline is not observed at stations 1 and 3 where active growth might have been expected. It is possible that our discrete depth sampling design missed a subsurface peak in biomass, but this is unlikely, since the 15-m sample is within the thermocline at these stations. At these stations the nutrients (nitrate and silicate) are depleted to a certain depth below the mixed layer, but within the euphotic zone, suggesting that at an earlier period active growth occurred at the base of the thermocline. Nutrient renewal by diffusion from below or regeneration does not appear to be sufficient to support a large biomass at the thermocline.

The species compositions at selected stations are shown in Table 1. The station that stands out is again that in the Gaspé current, at which a large proportion of the cells are diatoms, *Nitzschia delicatissima*, representing 60% of the total cell count. At the other stations diatoms represent a smaller percentage of the total, dinoflagellates and microflagellates being of relatively greater importance. *Prorocentrum* sp. dominated station 4 and was also present in appreciable amounts at stations 1, 3, and 8.

The estimates of average zooplankton biomass, calculated from single vertical net hauls, should not be influenced by diurnal vertical migration activity since the hauls were from near bottom to the surface. This assumption appears valid for the Anticosti gyre since little hourly variability in the estimates was observed. However, in the Gaspé current the hourly differences are considerably greater than the between station differences. The variability was due to temporal sampling in a patchy environment passing the station and/or some lateral movement during vertical migration of deep-water euphausid and *Calanus* spp. from the less productive gyre to the highly productive Gaspé current. The spatial and temporal species composition support this latter inference.

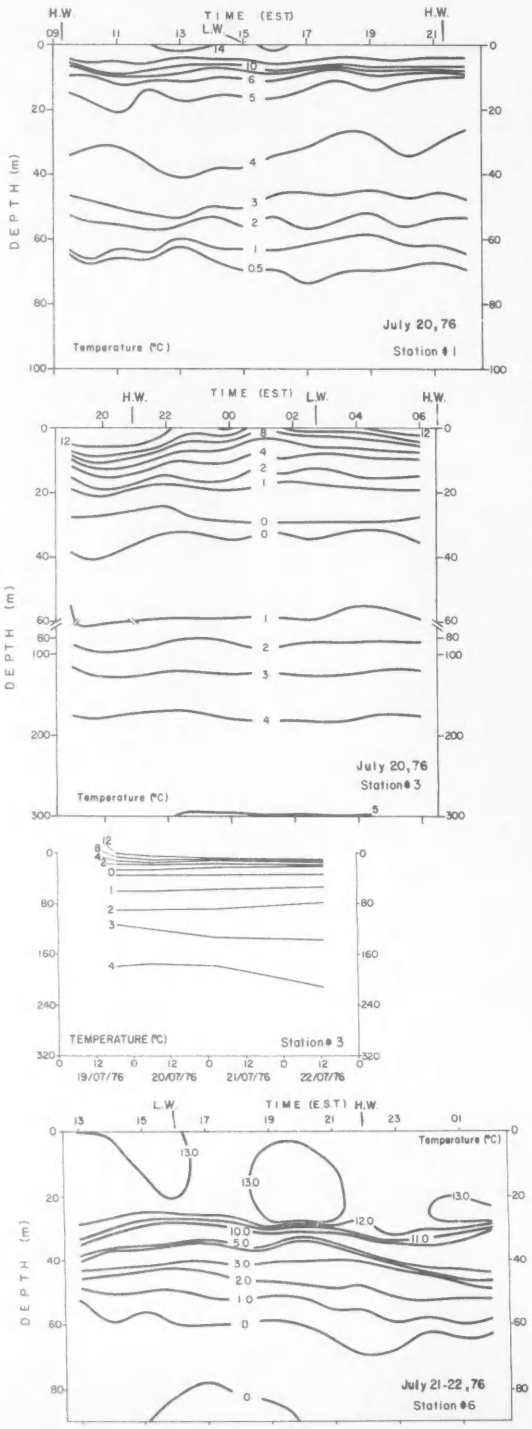
In biomass units, there is little difference between stations except for the Gaspé current station with perhaps marginally lower stock levels at the shallower stations (1, 7, 12). The zooplankton species composition, however, was very different in the shallow stations (1, 7, 12 and daytime hauls at station 6) relative to the deeper stations. The latter stations are characterized by small copepod species (*Acartia* spp. and *Pseudocalanus* spp.) and fish larvae (C. Lassus, Université du Québec à Rimouski, personal communication), whilst the deeper stations within the gyre are characterized by larger copepods (*Calanus* spp.), euphausid spp., and decapod larvae. As mentioned above there appears to be some mixing in the Gaspé current at night due to upwards and lateral migration of the gyre populations.

Short-Term Variability

In previous studies in the estuary (Sinclair 1978; Therriault and Lacroix 1976; Poulet et al. 1976), extreme short-term variability was observed in both the physical and biological measurements taken at a fixed station over a period of several hours to days. Such conditions complicate interpretation of spatial distributions. Because of this potential difficulty in interpretation of spatial structure (which is the primary aim of this study) and because of the lack of data on short-term variability in the study area, measurements of the parameters of interest were made over a tidal cycle at three of the stations (1, 3, 6) that were thought to cover the extremes in the environment. In addition station 3 was occupied 4 times over a period of 4 d.

TABLE 1. Species composition (%) at several of the stations.

Species	Stations (July 20–21, 1976)							
	1	3	4	6	6	7	8	12
Dinoflagellates								
—*Ceratium* sp. 1 (*fusus*)	0.02	.01	<.01	<.01	>.01	>.01	.06	.01
sp. 2 (*longipes*)	0.03	.02	<.01	<.01	>.01	.02	.05	.01
sp. 3	—	—	—	<.01	>.01	—	—	—
—*Dinophysis* sp. 1 (*norvegica*)	0.02	<.01	—	<.01	>.01	.01	.05	.02
sp. 2	0.02	<.01	—	<.01	>.01	.02	.06	—
—*Minuscula bipes*	.08	—	0.6	0.2	0.7	0.3	1.2	—
—*Peridinium* sp. 1	<.01	<.01	<.01	.01	>.01	—	—	>.01
sp. 2	—	.01	.01	.01	.01	.01	.03	.04
sp. 3	—	<.01	—	—	—	—	—	—
sp. 4	.02	—	—	.02	.01	—	—	.04
sp. 5	—	—	—	—	.01	—	—	—
sp. 6	—	—	—	—	—	—	—	—
sp. 7	—	—	—	—	>.01	—	—	—
sp. 8	—	—	—	—	—	—	—	.01
monospinum	.01	—	—	<.01	.01	—	—	.01
trochoidium	.01	2.5	—	.01	.03	.07	2.5	.04
—*Phalacroma rotundata*	<.01	—	<.01	—	>.01	>.01	.02	—
—*Prorocentrum* (sp.)	44.2	36.0	65.6	7.4	7.4	7.2	27.3	19.7
Total	45.1	38.4	66.2	7.7	8.17	8.28	31.27	19.88
Other flagellates								
—*Distephanus speculum*	—	—	—	<.01	—	—	>.01	>.01
—Flagellate #1	6.5	1.7	—	0.9	0.6	1.3	5.3	0.7
#2	0.4	—	2.9	0.6	0.5	0.9	1.5	3.5
#3	36.1	40.1	—	19.7	11.5	57.0	39.7	39.0
Total	43.0	41.8	2.9	21.2	12.6	59.2	46.5	43.2
Centric Diatoms								
—*Chaetoceros* sp. 1 (*pelagicus*)	—	—	—	<.01	>.01	—	—	—
sp. 2 (*laciniosus*)	.01	—	—	<.01	>.01	—	—	—
sp. 3	—	—	—	.01	>.01	—	—	—
sp. 4 (*similus*)	—	—	—	0.7	0.8	—	—	—
sp. 5	—	—	—	—	0.7	—	—	—
socialis	—	—	0.3	4.0	2.8	—	—	—
—*Coscinosira polychorda*	<.01	—	—	.02	.01	—	—	—
—*Leptocyclindrus* sp.	3.5	1.2	<.01	2.3	7.2	2.0	6.6	—
—*Rhizosolenia styliformis*	<.01	<.01	<.01	<.01	—	—	.01	—
sp. 1	<.01	—	—	<.01	>.01	—	.01	.01
fragilissima	—	—	—	—	0.5	—	—	—
—*Sheletonema costatum*	—	—	—	3.0	0.3	—	—	—
—*Thalassionema nitzchoides*	—	<.01	<.01	—	—	—	0.3	—
—*Thalassiosira nordenskioldii*	—	.02	—	—	—	—	—	—
sp.	<.01	—	—	.01	>.01	—	.03	.01
Subtotal	3.5	1.8	2.3	10.0	12.31	2.0	6.95	0.02
Pennate Diatoms								
—*Amphiprora* sp.	—	—	—	<.01	—	—	—	—
—*Amphora* sp.	<.01	—	—	—	—	—	—	—
—*Navicula* sp.	.34	—	—	.03	>.01	.01	2.0	—
—*Nitzchia closterium*	—	—	—	0.2	0.6	0.4	0.2	—
longissima	—	—	0.6	0.4	0.3	—	—	0.7
seriata	—	—	—	.01	—	—	—	—
delicatissima	7.7	18.0	27.3	62.0	65.5	—	11.3	35.8
Subtotal	8.7	18.0	28.5	62.6	67.0	0.5	17.5	36.5
Diatom Total	11.5	19.2	30.8	72.6	89.31	2.5	24.45	36.7
Other groups								
—Copepods	—	—	<.01	—	>.01	>.01	>.01	>.01
—Nauplii	<.01	<.01	.01	>.01	>.01	>.01	>.01	>.01
—Tintinnids	0.3	.03	0.1	.05	.06	0.1	1.8	0.3
Total	0.5	.03	0.1	.05	.06	0.1	1.8	0.3



The temporal isolines of temperature and silicate concentration (Fig. 7 and 8) at the three stations illustrate the differences in the stability. At station 1 there was no evidence of strong vertical oscillations in the water column and the advective transport past the station did not result in strong changes in the water characteristics, although a couple of surface pockets of warmer water (14°C) did pass the station. At station 3, in the center of the gyre, there was some shoaling of the isolines bringing colder water closer to the surface midway through the sampling period. However, there was no reflection of this in the nutrient distribution (Fig. 8) or in the chlorophyll distribution (Fig. 9). Also, the hydrological features showed considerable stability over the 4 d of sampling (Fig. 7). In contrast to the relative stability in hydrological, nutrient, and phytoplankton biomass at station 1 and 3, there was considerable variability in the Gaspé current measurements. The temperature inversions observed in the surface water indicate turbulent flow and the silicate isolines (which show a similar behavior as the other nutrients) suggest entrainment perhaps related to the rising tide. A longer time series would be necessary, however, to adequately describe tidal effects. The corresponding short-term variability in the phytoplankton biomass in the Gaspé current relative to the gyre and north shore are shown in Fig. 9.

In sum, there is a relative constancy (on a scale of hours) in this environment, except for the Gaspé current. This, and our observations of the temperature isolines over the 4 d at station 3 (Fig. 7) and the constancy in the features observed during the repetition of the north–south transect over a couple of days, suggest that we are not observing an ephemeral phenomenon. The circulation pattern in the northwestern Gulf, the gyre, and the Gaspé current, are characterized by specific hydrological conditions that permit different levels of phytoplankton production and species composition. Also, single observations made at a sampling station in this broad area, except in the Gaspé current, are probably representative of conditions over at least several days.

Derived Parameters

The derived parameters (P/B and C/chlor ratios) were estimated systematically at each station at 5 m depth to try to evaluate the physiological state of the mixed phytoplankton assemblages. To aid in the interpretation of these parameters on the horizontal plane, their variability with depth and their short-term variability were analyzed at three of the stations (1, 3, 6). If complications such as diurnal variability are ignored for the initial evaluation, higher values of growth rate and assimilation index (chlorophyll-normalized, light-

FIG. 7. Temperature isolines observed over a short time period at stations 1, 3, and 6.

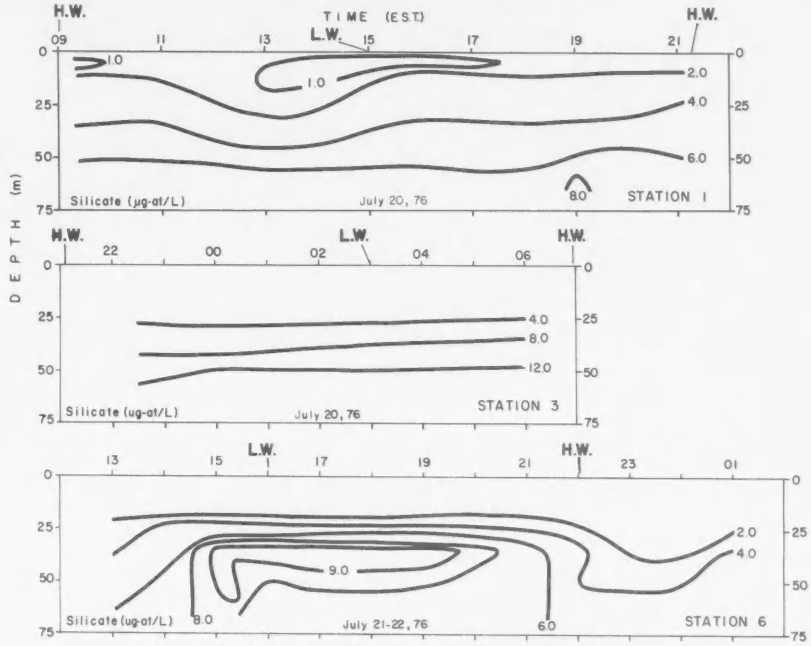


FIG. 8. Silicate isolines observed over a short time period at stations 1, 3 and 6.

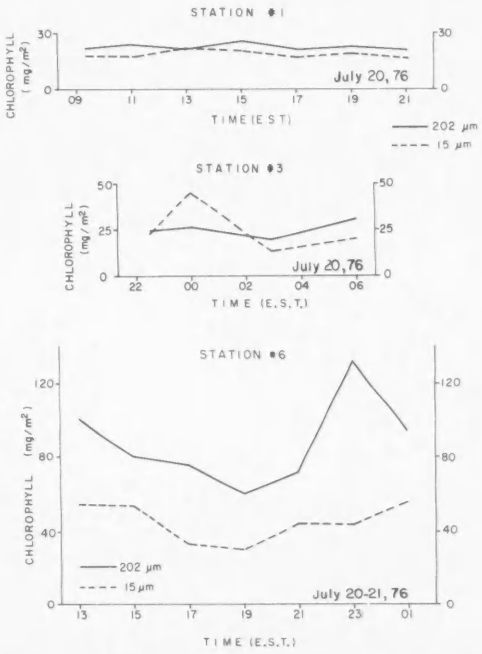


FIG. 9. Short-term variability in water column chlorophyll (in two size categories) observed at stations 1, 3, and 6.

saturated photosynthesis) were observed at stations 1 and 4 on the north–south transect and at stations 7, 8 on the east–west transect (Fig. 10). It was at stations 4 and 8, which have a strong *Prorocentrum* sp. component, that relatively higher nutrient concentrations were observed in the surface layer (Fig. 2 and 3). It is possible then that the higher P/B values and low C/chlor indicate improved physiological state in response to the higher nutrient concentrations or nutrient flux (stations 1 and 7). The C/chlor ratio is systematically lower in the stations within the gyre in which good physiological state is indicated (1, 4, 7, 8, 11). Although there are taxonomic as well as environmental differences in this ratio, with a given species composition one would expect lower values to be associated with good physiological state (Eppley 1972). It is of interest that the nannoplankton dominated the production, as was the case for the biomass, rather systematically in both transects.

The low values of the P/B ratios and the high C/chlor ratio observed at station 6 in the Gaspé current are not representative of the "average" situation since the P/B values observed during the 12-h fixed station are in general high, with a large portion of the growth contributed by the net plankton fraction (Fig. 11). Also, the C/chlor ratio is consistently low, which is characteristic of actively growing diatoms, at the fixed station. The derived parameters at station 6, indicated in Fig. 10, were measured during the second occupation of this transect during which relatively

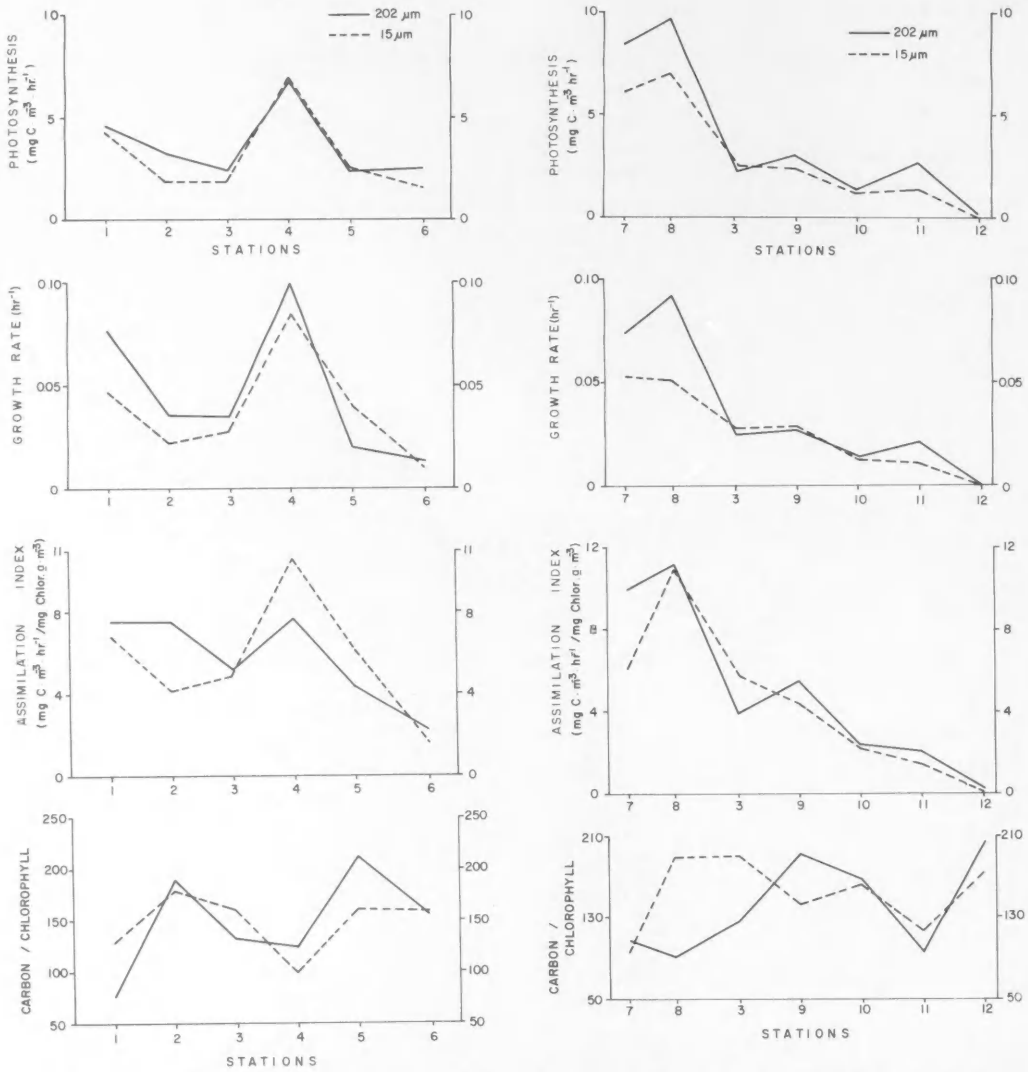


FIG. 10. Photosynthesis at saturating light intensity and the derived parameters, estimated for two size categories at 5 m depth at stations across (a) the north–south transect and (b) the east–west transect .

lower biomass was observed, and nannoplankton dominated (Fig. 5 middle graph).

The considerable short-term variability in the derived parameters observed during the fixed station (Fig. 11 and 12) complicated the interpretation of the above spatial pattern. However, if the means are considered, tendencies are discernable (Fig. 13). The water column chlorophyll *a* and light-saturated primary production at 5 m depth are indicated in the two upper boxes, and the higher productivity and net plankton contribution of the Gaspé current station are clearly evident. Both the nearshore stations have higher assimilation indices and growth rates, but the north shore station at which diatoms are not important is also characterized by a high average C/chlor ratio. This combination of high assimilation index and C/chlor ratio observed at station 1 has been observed to be characteristic of chrysophyte flagellates, presumably in good physiological condition in the lower St. Lawrence Estuary (Sinclair 1978). Flagellates are an important component of the biomass

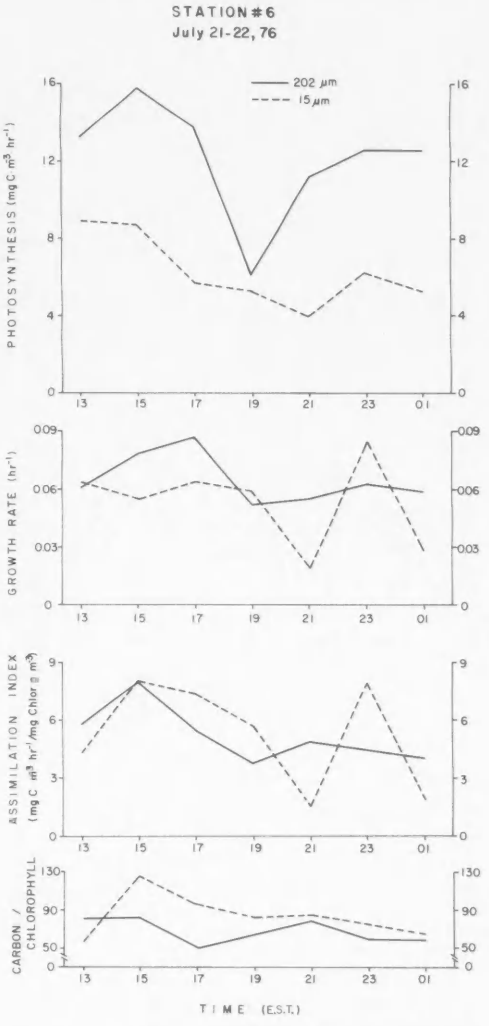


FIG. 11. Short-term variability in photosynthesis at saturating light intensity, and the derived parameters, estimated for size categories at 5 m depth at station 6.

at station 1 (43%, Table 1). Also the combination of high growth rate, average assimilation ratio, and low C/chlor ratio was characteristic of diatoms, as is observed in this study at station 6.

The distribution of the derived parameters with depth at station 3, central gyre, suggest that the physiological condition improves with depth, the P/B ratios increasing and the carbon/chlorophyll ratio decreasing (Fig. 14). Nutrient availability by upward diffusion, or due to directed movement by flagellates, would be higher closer to the thermocline and is consistent with the derived parameter distributions. At station 6 (Fig. 14) the contribution of netplankton photosynthesis to the total production decreases with depth as was noted above for the biomass contribution. It is of interest that the physiological condition (as indicated by the derived parameters) of the mixed phytoplankton populations is vigorous throughout the mixed layer to the base of the relatively deep pycnoline (~60 m in Fig. 4), which is considerably deeper than the 1% light level. This suggests that active vertical mixing within the surface mixed layer permits an average light regime that is sufficient to maintain vigorous populations throughout the mixed layer. The chlorophyll *a* profile, however, suggests that vertical mixing is not sufficiently intense to disperse the light-induced depth gradient in chlorophyll. Another explanation of the good physiological condition with depth is suggested by the separtion of the species assemblage with depth. The low biomass deeper water within the mixed layer is dominated by smaller cells, presumably flagellated, which have some capacity for directed vertical movement in response to the nutrient and light gradients. The derived parameters for the <15-μm size fraction, which includes the flagellates in question, are essentially constant with depth while the <202-μm values fluctuate strongly. It is possible then that the smaller cells can maintain their physiological condition through vertical migration without the constraint of an active vertical mixing by turbulent diffusion.

General Discussion

Although our observations are limited to a single midsummer period, and a few sampling stations, some conclusions can be tentatively drawn concerning the relationship between the circulation pattern and plankton distributions in the northwestern Gulf. The gyre forms a relatively stable environment within which there is considerable uniformity in plankton species composition and trophic level biomasses. The upwelling results in a shallow mixed layer but the degree of density stratification during the midsummer months appears to prevent large-scale nutrient enrichment of the surface mixed layer. At a few central stations (4, 8, 11) there was some evidence of nutrient enrichment both from the nutrient values themselves and the apparent physiological state of the mixed species assemblages. But the species composition at these stations was similar to the other deep-water stations, consisting of dinoflagellates and microflagellates, suggesting that the enrichment is a minor and perhaps short-lived event. One might expect a major nutrient enrichment event to be associated with diatoms and presence of "net plankton" (Malone 1971). The phytoplankton species composition and/or physiological state indices observed at the shallover nearshore stations (1, 6, 7) also indicate relatively rapid nutrient turnover in the surface mixed layer even though low values of nitrate and silicate are observed. Stations 1 and 7 are characterized by flagellates in good

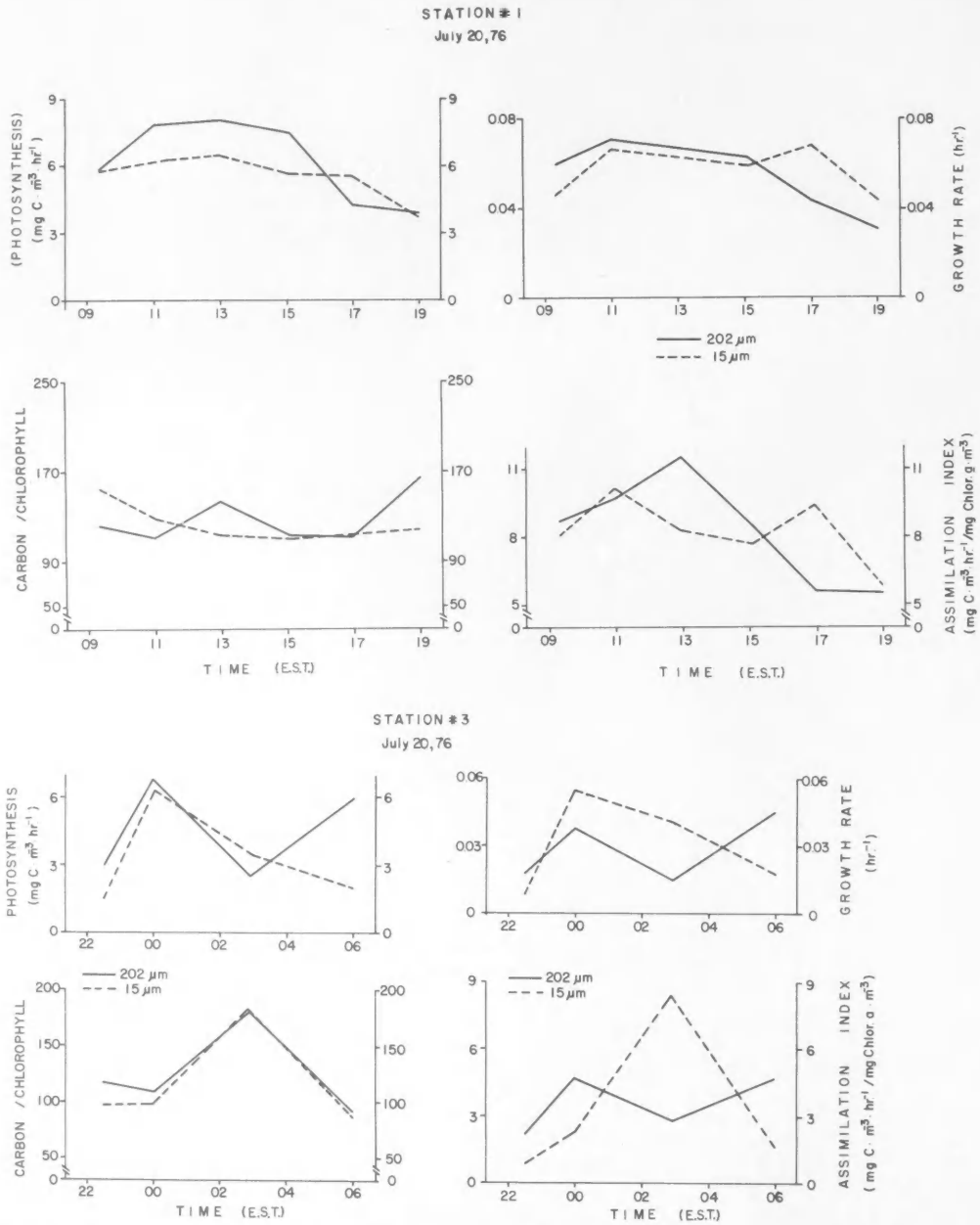


FIG. 12. Short-term variability in photosynthesis at saturating light intensity, and the derived parameters, estimated for size categories at 5 m depth at stations 1 and 3.

physiological state and the Gaspé current station by a high biomass, an important contribution by diatoms and high average growth rates. This latter combination suggests active nutrient sources through entrainment and/or horizontal advection. Without data from upstream stations within the Gaspé current it is not pos-

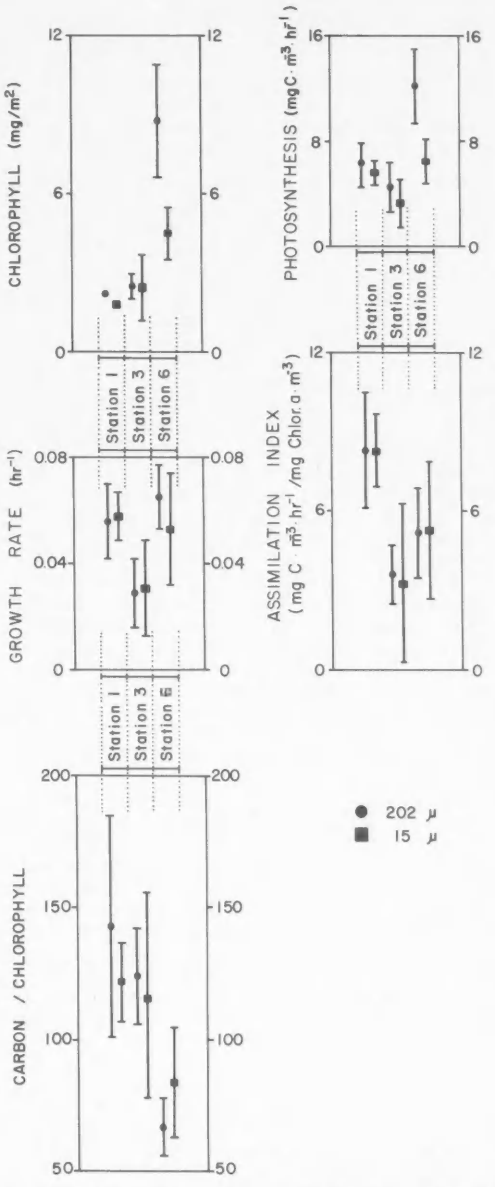


FIG. 13. Mean water column chlorophyll *a* and light-saturated photosynthesis along with the derived parameters estimated for two size categories at 5 m depth, at stations 1, 3, and 6. The vertical bars represent SD. Means were calculated from the fixed station estimates.

sible to evaluate even qualitatively the relative importance of the two sources, but the result of nutrient enrichment is clear from the species composition and physiological state.

The general features of the phytoplankton production cycles within the northwestern Gulf can be inferred from this study and a subsequent spring cruise. During the last week of April 1977 a massive spring bloom was observed throughout the area with considerable uniformity in species composition (*Thalassiosira* spp., *Biddulphia* spp., *Nitschia* spp.) both within the gyre and the Gaspé current. Subsequently, from this study, one can infer a classical temperate latitude nutrient-limited reduction in primary production within the gyre over the summer months and a continuation of high production within the Gaspé current due to the continual nutrient enrichment.

Since the zooplankton species composition varies in parallel with the level of summer productivity, it is tempting to conclude that we are observing two semi-enclosed pelagic ecosystems — the Gaspé current characterized by continuous high summer production, large-celled diatoms, small copepods, and fish larvae; and the Anticosti gyre characterized by nutrient-limited phytoplankton growth, small flagellates, large copepod species, euphausids, and decapod larvae. It is, however, probably a depth-induced effect rather than observation of a community structure induced by different levels of production and cell size, since at stations 1 and 7 the intermediate situation is observed (small flagellates and small copepods). Nevertheless, due to the cell sizes and herbivore sizes the phytoplankton–zooplankton link should be quite different between the Gaspé current and the gyre.

Our results do not agree with Steven's (1974) generalization that the northwestern Gulf is in a spatial sense uniformly productive over the summer months and also the absolute levels of primary production that are reported. The Gaspé current does appear to be highly productive during the summer months, but the major proportion of the study area was observed to have a low phytoplankton biomass and to be nutrient limited (to a varying degree spatially). From the surface chlorophyll values and typical radiation levels for July at this latitude, order of magnitude estimates of daily production can be made. In Table 2 estimates of daily water column primary production are made following the methods of Ryther and Yentsch (1957). Estimate 1 assumes that chlorophyll *a* is uniformly distributed with depth at a concentration equal to the surface value. In the second method, daily photosynthesis is estimated at several depths in relation to the specific chlorophyll *a* depth distribution, and water

TABLE 2. Estimates of daily primary production (mg $C \cdot m^{-2} \cdot d^{-1}$) at stations 3 and 6.

	Station 3	Station 6
Estimate 1	73	540
Estimate 2	79	450
Steven (1974)	~5000	~5000

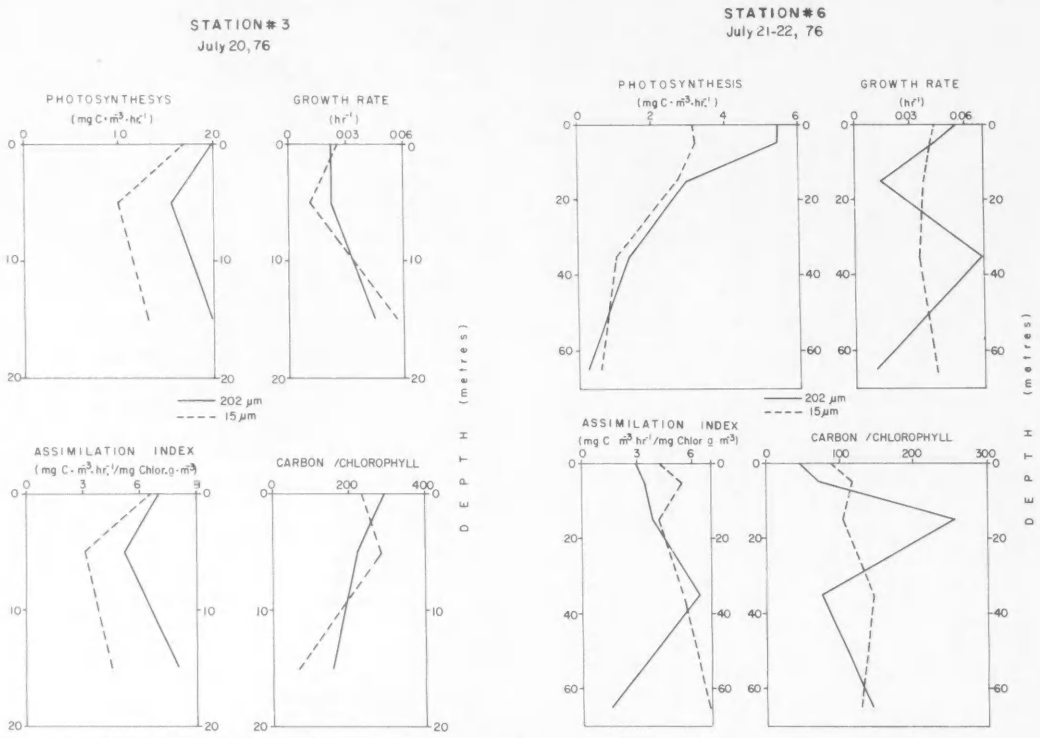


FIG. 14. Vertical distribution of light-saturated photosynthesis, and the derived parameters, estimated for two size categories at stations 3 and 6.

column daily photosynthesis calculated by graphic integration. The two estimates are similar, but are one to two orders of magnitude less than the values reported by Steven (1974). A similar difference in daily production was noted between Steven's and our laboratory for the lower St. Lawrence Estuary (Sinclair et al. 1976).

There is considerable interest in accurate primary production estimates in this area. For example, some estimates of seasonal photosynthesis rates and their spatial variability are necessary to predict the effect of changes in freshwater runoff on nutrient budgets by the construction of dams along the north shore. This study, which describes the relationship between the circulation patterns and plankton distributions, suggests that the region cannot be characterized by a single level of production and that the absolute values for any one region are in doubt. The Gaspé current, characterized by high primary production over a 6-mo period, should have an important ecological impact on the higher levels in the food chain both within and around the current. The suggested lateral migration of euphausid and *Calanus* spp. populations into the current at night and the abundance of feeding herring populations (Messieh 1978) support this supposition.

Acknowledgments

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The Intrusion of Fine Sediments into a Stable Gravel Bed

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BESCHTA, R. L., AND W. L. JACKSON. 1979. The intrusion of fine sediments into a stable gravel bed. J. Fish. Res. Board Can. 36: 204–210.

A rectangular flume was used to study variables affecting the intrusion of fine sands into a stable gravel streambed. The amount of intrusion by sand (median particle diameter 0.5 mm) was determined under varied conditions of discharge, depth, velocity, flume slope, and rates of sediment transport. During all experimental tests, sand particles were trapped in voids within the upper 10 cm of an initially clean gravel bed (median particle diameter 15 mm), forming a barrier to further intrusion. An analysis of flow variables showed that flow conditions, as indexed by Froude number, significantly (90% confidence level) affected intrusion amounts, possibly by influencing the rate and depth of formation of the sand seal. Intrusion amounts, expressed as a percent of total volume, varied from 2 to 8%. Two replications used a finer grade sand (median particle diameter 0.2 mm) that intruded more and, in one case, completely filled the gravel pore space (25% by volume), further indicating that particle size, and not hydraulic variables, may have a more important influence on the total amount of intrusion.

Key words: sediment transport, intrusion, streambed, substrates, riffles, sedimentation

BESCHTA, R. L., AND W. L. JACKSON. 1979. The intrusion of fine sediments into a stable gravel bed. J. Fish. Res. Board Can. 36: 204–210.

Nous nous sommes servi d'une auge rectangulaire pour étudier les variables affectant l'intrusion de sable fin dans le lit de gravier stable d'un cours d'eau. Nous avons déterminé les quantités de sable (diamètre médian des particules 0,5 mm) introduites dans diverses conditions de débit, profondeur, vélocité, pente de l'auge et vitesse de transport des sédiments. Pendant tous les essais, les particules de sable furent emprisonnées dans les espaces des 10 cm supérieurs d'un lit de gravier initialement propre (diamètre médian des particules 15 mm), formant une barrière à l'introduction d'une plus grande quantité de sable. Une analyse des variables du débit démontre que ce dernier, indexé selon le nombre de Froude, affecte de façon significative (limite de confiance à 90%) les quantités introduites, possiblement en agissant sur la vitesse et la profondeur auxquelles la couche scellante se forme. Les quantités introduites, exprimées en pourcentage du volume total, varient de 2 à 8%. Dans deux essais répétés, nous avons utilisé un sable plus fin (diamètre médian des particules 0,2 mm) qui pénétra davantage et qui, dans un cas, remplit complètement les pores du gravier (25% en volume). C'est une indication de plus que la taille des particules peut avoir une plus grande influence que les variables hydrauliques sur le degré d'intrusion.

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IN small mountain streams, particle size distributions of bed materials are affected by many interrelated factors. Climate, soils, parent materials, watershed characteristics, and land use activities all interact to produce various combinations of sediment sizes. Particularly important are the bed materials approximately 1 mm in diameter or smaller. These fine sediments can significantly affect fish habitat and other instream biota (Gibbons and Salo 1973; Meehan and Swanston 1977).

Although the evidence is not entirely consistent, research results have generally shown that increased levels of fines have detrimental biological effects (N. H. Ringler unpublished data; Moring and Lantz 1974; Iwamoto et al. 1978). In forest streams of the Pacific Northwest, fine sediments can affect anadromous and resident fish populations after the deposition of eggs. Fine sediments may reduce intergravel circulation of water, which decreases the dissolved oxygen available in the spawning gravels (Vaux 1962). Apparently, relatively small percentage increases in the volume of fine sediments may greatly reduce the permeability of stream gravels (Phillips 1971). In addition, sediments may block the movement of fry from the gravels to the stream (Koski 1972). By eliminating intergravel crevices for juvenile fish and other organisms, fine sedi-

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ments alter habitats and decrease fry survival. Iwamoto et al. (1978) identified several studies noting such effects. Furthermore, many common riffle insects are unable to move upstream on sand substrates (Luedtke and Brusven 1976).

Research has also shown that forest harvesting operations (including road building, yarding, and slash disposal) in steep terrain can increase the amount of sediments entering the stream system. Although watershed responses to land use are highly variable, sediment transport rates typically recover, with time, toward pretreatment levels (Anderson 1972; Brown 1972; Megahan 1974; Swanson and Dyrness 1975). The linkage between this temporary increase in sediment production and changes in streambed composition have not been identified.

Unfortunately, little information is available relating the temporal and spatial variability of percent fines in streambed gravels to hydraulic variables, rates, and mechanisms of sediment transport (i.e. suspended load vs. bedload), as well as sediment characteristics. Although sediment transport mechanisms are complex (Lawson and O'Neill 1975), selected hydraulic and sediment variables may be useful for characterizing these mechanisms and the intrusion or deposition of fines.

This study evaluates factors affecting the intrusion of fine sediments into a stable gravel substrate, thereby improving understanding of the process and possible biological impacts.

Methods

The study was conducted during the summer of 1977 at the Kalama Springs Field Laboratory of the Weyerhaeuser Company, approximately 80 km east of Longview, Washington. A wooden flume, 7.6×0.71 m, was constructed so that a range of streamflow and sediment transport conditions could be evaluated. Although a flume does not simulate the exact conditions found in mountain streams, this approach was used because flow conditions and sediment transport variables could be controlled and modified as desired. The flume was hinged at the upstream end and supported by hydraulic jacks so the bottom slope could be adjusted. Several glass windows installed along the sides of the flume allowed observations of both the transport and intrusion of fine sediments.

Water from Kalama Springs was used for each test. A headgate upstream from the flume controlled the discharge of water into an artificial channel that led to the flume. At the immediate upstream edge of the flume, a weir and staff gage enabled us to monitor discharge during each test. Water temperatures remained a constant 7°C throughout the study.

A fertilizer hopper 46 cm wide was mounted at the upstream end of the flume to meter fine sediments into the flowing water. Delivery rates of fine sediments were controlled by a calibrated lever that opened the spreader. After each test, actual rates of sediment delivery or inputs were calculated from the volume of fines that had passed through the spreader.

Clean gravels to a depth of 30.5 cm were placed into the test section of the flume for each test. These gravels, leveled and lightly tamped to provide a uniform gravel surface, averaged 15 mm diam and had a particle size distribution as shown in Fig. 1. The particle density and porosity of these gravels averaged 2.67 g/cm³ and 35% (by volume). Sand-size sediments, averaging 0.5 mm diam (Fig. 1) and a particle density of 2.26 g/cm³, were used for most of the intrusion evaluations. Both sands and gravels match those being used by fisheries biologists at the Weyerhaeuser Company to assess survival to emergence ratios of salmonid embryos in artificial redds (J. Fisher, Weyerhaeuser Company, Longview, Wash., personal communication, 1977). The relatively low particle density of the 0.5-mm sands can be attributed to the high occurrence of pyroclastic rock types comprising individual sand grains.

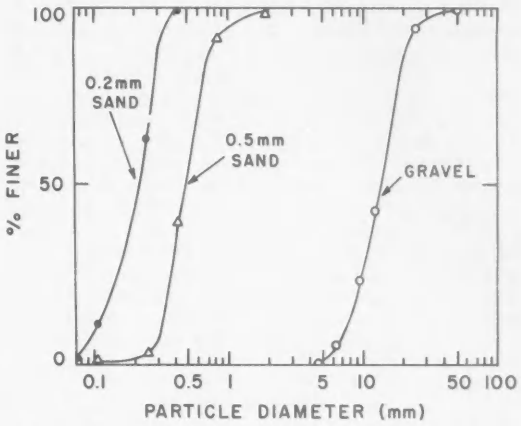

FIG. 1. Size distributions of sands and gravels used to evaluate the intrusion of fine sediments into a gravel-bedded test channel.

During 18 separate runs in the test flume, we evaluated these sediment transport conditions: 1) three discharge rates (≈50, 75, and 90 L/s); 2) three slope conditions (Froude numbers ≈0.6, 1.1, and 1.5); and 3) two (low and high) sediment input rates (≈2500 and 11 500 g/min). These 18 tests ($3 \times 3 \times 2 = 18$) were conducted with the 0.5-mm sand. Two additional tests used fine sediments with a particle diameter averaging 0.2 mm (Fig. 1) and a density of 2.53 g/cm³. A final run for an extended period (i.e. 120 min) was made with the 0.5-mm sand at the low input rate to determine maximum levels of intrusion for a given flow condition. All other tests lasted either 30 or 60 min for the high and low input rates, respectively, because field observations and measurements during individual tests indicated that most of the intrusion occurred within the first 15–20 min of a run.

For a given discharge rate, flume slope was adjusted for evaluating a range of Froude numbers. The dimensionless Froude number (F_r), representing the ratio of inertial to gravitational forces in fluid flow (Streeter and Wylie 1975), is expressed as

$$F_r = \frac{\bar{V}^2}{gy}$$

where $\bar{V}$ = mean velocity, m/s
g = acceleration due to gravity, 9.8 m/s²
y = depth of flow, m

The Froude number is a useful way to characterize flow conditions. For subcritical flow ($F_r < 1.0$), stream conditions are typified by relatively deep, slow streamflow. At a critical flow ($F_r = 1.0$), the specific energy ($E = \bar{V}^2/2g + y$) is at a minimum. "Standing waves" in a stream indicate critical flow conditions. Supercritical flow ($F_r > 1.0$) is characterized by relatively shallow, rapid streamflow. Because all three conditions occur in mountain streams, this study evaluated a range of Froude numbers.

For each test run, water velocity and depth were measured at 5-cm intervals across the width of the flume near the center of the gravel test section. Velocities were measured using a pygmy current meter. Vertical velocity profiles were also determined for each flow condition. Two small siphon tubes (0.64 mm diam) extracted water and transported sediment from elevations of 1 and 6 cm above the gravels to determine sediment concentrations immediately above the gravel bed.

After each run, cores of the gravel bed were frozen (Walkotten 1973) for estimating the amount of sand that had intruded into the test gravels. This procedure was also intended to provide information about the positioning of intruded sands within the gravel bed. However, during the first several experimental runs, we had a problem — inserting the probe of the freeze-core sampler disturbed the gravels so that sands deposited among the surface gravels moved downward and were redeposited. Wendling (1978) encountered a similar problem when sampling streambed sediments in Alaskan streams. In our situation, the retrieved frozen core usually had sands concentrated within several centimeters of the flume bottom but few, if any, sand particles above that point. Observations through the flume windows confirmed that the sands settled throughout the gravel profile during sampling. Consequently, after the first three tests (no. 1, 4, and 6), we implemented another method of sampling. We buried two containers (12.5 cm diam, 15.0 cm tall, and open at the top) in the gravels before a run, removed them after completing the run, and then determined the dry weight of sands.

Total intrusion for a given run was calculated by dividing the weight of the sands in each container by the weight of the gravels in a column 12.5 cm diam and 15.0 cm high (even though the total depth of gravels was 30.5 cm). These weights were adjusted to account for differences in particle densities between the sands and gravels. Thus, intrusion amounts were expressed as percent by volume of intruded sands in relation to the total volume of sample. The choice of a 15-cm depth was arbitrary, but made the calculated percentages more representative of average fine concentrations in the gravels.

After each run and the retrieval of sampling containers, the gravels were flushed from the flume and clean gravels were inserted into the test reach.

Results and Discussion

The flume windows proved particularly useful for visually observing sediment transport and intrusion processes. At low Froude numbers ($F_r < 0.9$), sands were transported mostly within 1 cm of the gravel bed surface. The transport process consisted mainly of individual grains rolling and sliding along the tops of gravels and other sand particles. At Froude numbers generally greater than 0.9, transport mechanisms shifted so that the suspension and saltation of sand particles became increasingly important. This was further illustrated by the increased average sediment concentrations (mg/L) measured at both 1 and 6 cm above the gravel bed during runs with 0.5-mm sands. Sediment

	Froude number		
Height (cm)	$0.5 < F_r < 0.9$	$0.9 < F_r < 1.3$	$1.3 < F_r < 2.6$
1	1700	4800	4500
6	120	370	760

concentrations 6 cm above the gravels typically averaged about one tenth of those measured at 1 cm. Dunes, ripples, or other bed forms were not observed during any of the test runs.

Apparently sediment deposition and intrusion into the gravels involves two principal mechanisms: 1) the transport and deposition of sand particles into the surface voids of the gravel bed, and 2) the settling of the particles into deeper gravel voids. The settling process occurs primarily under the influence of gravitational forces, but seems assisted by turbulent pulses at the gravel surface.

At low Froude numbers ($F_r < 0.9$), the 0.5-mm sands quickly established a sand "seal" within the upper 5 cm of the test gravels as the larger sand particles bridged the openings between adjacent gravel particles and prevented the downward movement of additional sands into the gravels. Once this sand seal had formed and the intergravel space above had filled with fines, the intrusion process stopped. Additional sands were transported past the gravel test section. At higher Froude numbers ($F_r > 0.9$), flow characteristics began to alter this process. Observations indicated that most deposition and intrusion now occurred within the upper 5–10 cm of the gravels. Because of the higher velocities associated with higher Froude numbers, these flows were characterized by greater bed shear and the periodic jiggling of the surface gravels. These turbulent pulses seemed to inhibit the formation of a sand seal near the gravel surface. As a result, the sand seal still formed, but generally deeper within the test gravels. Again the formation of a sand seal in a stable gravel substrate would preclude the additional intrusion of fine sediments into the lower gravels.

Table 1 summarizes measurements for each of the 21 experimental runs. For the first 18 runs (using the 0.5-mm sands), an analysis of variance indicated that flow condition, as indexed by Froude number, significantly (90% confidence level) influenced intrusion amounts. Apparently the main influence of flow conditions was to vary, within a relatively narrow range, the depth and rate of formation of the sand seal. Other flow indicators, such as average bed shear stress, unit stream power, or Reynolds number, did not significantly affect the amount of intrusion, making a physical interpretation of the Froude number relationship difficult.

TABLE 1. Test conditions during the intrusion of fine sediments into a gravel-bedded channel.

Test	Mean particle size (mm)	Test duration (min)	Hydraulic variables				Sediment transport and intrusion variables			
			Discharge (L/s)	Froude number	Velocity (m/s)	Depth (cm)	Input rate (g/min)	c_1[a] (mg/L)	c_6[a] (mg/L)	Total intrusion (% by vol)
1	0.5	60	45.3	0.66	0.75	8.6	1 680	1 010	13	—
2	0.5	60	49.8	1.13	0.92	7.6	2 470	1 430	35	4.6
3	0.5	60	51.0	1.55	1.03	7.0	1 970	1 440	—	6.6
4	0.5	30	44.2	0.89	0.82	7.6	10 000	2 200	72	—
5	0.5	30	53.8	1.08	0.94	8.2	12 850	10 980	260	8.0
6	0.5	30	48.7	2.57	1.20	5.7	8 570	3 770	—	—
7	0.5	60	78.7	0.54	0.84	13.2	1 990	790	70	7.0
8	0.5	60	73.1	1.28	1.09	9.5	2 600	1 500	120	4.5
9	0.5	60	70.2	1.52	1.14	8.7	2 470	1 770	170	6.8
10	0.5	30	73.1	0.53	0.81	12.7	11 860	2 800	200	5.3
11	0.5	30	73.6	1.18	1.06	9.8	11 860	7 210	660	5.8
12	0.5	30	72.2	1.53	1.15	8.9	12 920	7 480	1 200	8.3
13	0.5	60	90.1	0.56	0.88	14.3	2 140	812	70	5.9
14	0.5	60	90.6	1.06	1.10	11.6	3 630	2 450	230	5.0
15	0.5	60	96.3	1.43	1.24	11.1	3 130	1 750	500	7.4
16	0.5	30	91.8	0.66	0.94	13.7	10 830	2 470	280	2.6
17	0.5	30	92.3	0.92	1.05	12.4	11 860	8 970	1 100	5.8
18	0.5	30	92.6	1.52	1.24	10.5	11 860	10 740	1 150	10.4
19	0.2	60	94.0	0.63	0.93	14.1	2 150	970	210	24.9
20	0.2	60	91.5	1.12	1.12	11.5	1 980	860	220	15.3
21	0.5	120	91.8	0.93	1.06	12.2	3 220	1 370	160	6.5

[a] c_1 and c_6 = sediment concentrations 1 and 6 cm, respectively, above gravel surface.

Intrusion amounts by total volume ranged from 2 to 8%.

An increasing Froude number affected intrusion amounts differently at low (Fig. 2) and high (Fig. 3) rates of sediment input. At low sediment input rates, and hence low transport rates across the gravels, intrusion amounts reached a minimum for Froude numbers of approximately 1.0–1.2. However, at high rates of sediment input of the 0.5-mm sand, intrusion amounts generally increased as Froude number increased. These contrasting results further indicated that the intrusion of fines into a stable gravel bed is a complicated and not fully understood process.

Although only 2 of the 21 runs (no. 19 and 20) used 0.2-mm sands, these runs substantially contrasted with those using 0.5-mm sands. The major difference was the absence of a sand seal in the upper gravels. Instead, these finer sands generally moved down through the gravels by gravity and began to fill the test gravels from the bottom up. These observations concur with flume studies conducted by Einstein (1968) where he evaluated the intrusion of silt-size particles into streambed gravels and concluded that silts fill gravels from the bottom upwards.

In comparison to the 0.5-mm sands, more 0.2-mm sands intruded (Fig. 2), further suggesting that particle size is an important variable affecting intrusion into stable gravels. The amount of intrusion by the 0.2-mm sands also substantially decreased as Froude number increased from 0.6 to 1.1. The percentages shown in Fig. 2 are the results of tests that lasted only 1 h. Yet, within that time frame, the 0.2-mm sediments attained intrusion amounts from 15 to 25%. Complete filling of the gravel interstices would be represented by a value of approximately 25% by volume for the 0.2-mm sands.

Although observations indicated that the intrusion and deposition of 0.5-mm sands stabilized before completion of a given run, test no. 21 was implemented to see whether intrusion would continue over a longer period. The measured intrusion of 6.5% for this run was within one percentage unit of that predicted from the curve in Fig. 2. This result seems to substantiate observations that intrusion had essentially stopped by the end of each run.

Particle size distributions for samples obtained during run no. 21 provide an interesting contrast to the transport and intrusion of fine sediment. The particle size distribution of sands from the siphon located 1 cm above the gravels is essentially identical to that of the 0.5-mm sands added at the upstream end of the flume. However, the intruded sands have a mean particle size of <0.3 mm, indicating that the intrusion process into a stable streambed may be selective towards smaller particles (Fig. 4).

Results also illustrate that the mean particle size of sediment in transport 6 cm above the gravels is finer than at 1 cm (Fig. 4). Steep velocity gradients near the gravel surface (Fig. 5) were capable of temporarily

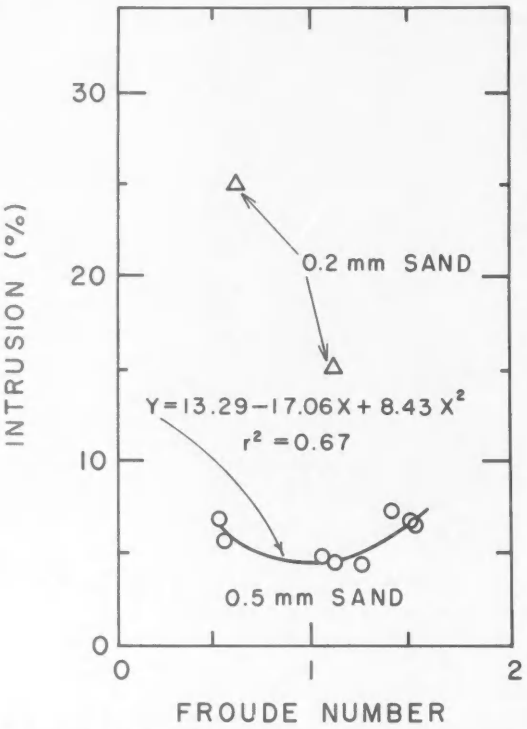

FIG. 2. Total intrusion of sand, percent by volume, in relation to Froude number for low sediment input (≈2500 g/min).

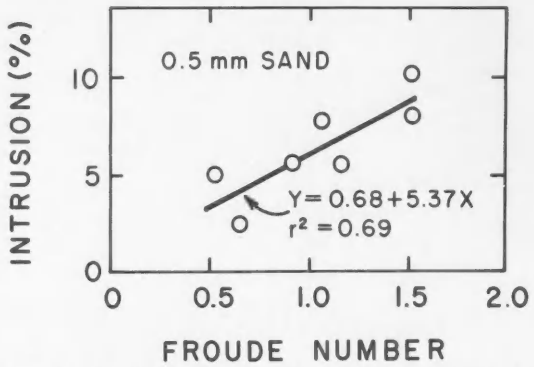

FIG. 3. Total intrusion of sands, percent by volume, in relation to Froude number for high sediment input (≈11 500 g/min).

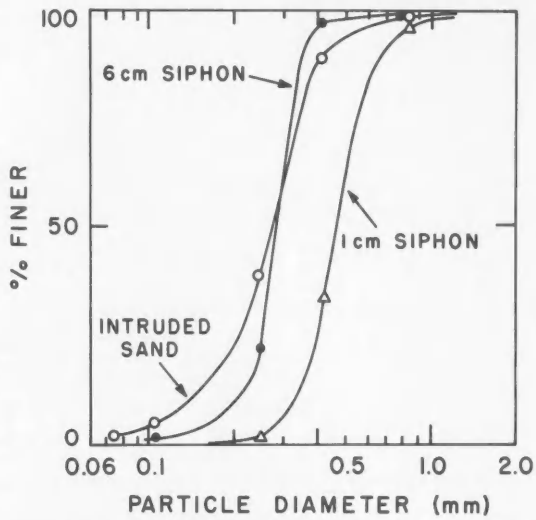

FIG. 4. Size distributions of sands during intrusion test no. 21.

suspending nearly all sand particles at least 1 cm above the bed. At the 6-cm elevation, a reduced velocity gradient and resultant shear stresses (even though average velocities are higher) reduced the transport of larger sediment particles. This is illustrated by the shift in mean particle sizes from 0.5 to 0.3 mm at elevations of 1 and 6 cm, respectively, above the gravels.

An additional experimental run with a flow of approximately 90 L/s and a Froude number exceeding 1.0 was made at the Kalama Springs Field Laboratory; results were not quantified. The 0.5-mm sands intruded until a sand seal formed and the interstices of the gravels had filled with sand. At that point, we stopped adding sand and allowed the flow to clean or flush the fines from the gravels. Observations and photographic evidence showed that fines flushed from the gravels to a depth of about 1 cm. Sands below 1 cm were not entrained by the flowing water, and further flushing did not occur, suggesting stream gravels can be cleansed or flushed only when in motion during high flow events. Without movement of these gravels, apparently no mechanisms are available for flushing. Thus the amount of fines transported during high flow events may have a pronounced effect on the natural quality or composition of gravel substrates in mountain streams. However, McNeil and Ahnell (1964) reported that local fines can be removed from the streambed during spawning.

Flow conditions, sediment transport rates, and sediment particle size all influenced the amount of fines deposited in initially clean gravels. These results indicate the need for improved understanding of the mechanisms of intrusion and the importance of mean particle size in affecting the intrusion of fines. Although several factors were found to influence intrusion, the direct application of these results to natural streams is tenuous. Measurements in small mountain streams must be improved before the ramifications of flume studies

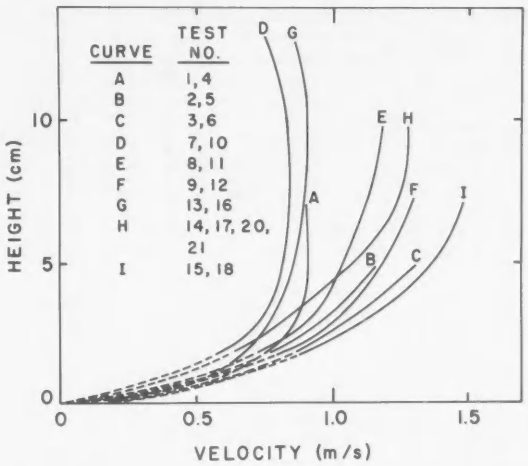

FIG. 5. Velocity profiles measured above gravel test section.

can be fully understood and extrapolated to field situations. For example, flow conditions and transport rates of fine sediments over known spawning gravels must be characterized for a range of high flow conditions. With such information, we may be more able to fully assess the impacts of sedimentation upon the habitats and organisms found in mountain streams.

Even though the quantitative results of this study cannot be directly applied to small streams, the study has several implications concerning land-use activities and stream sedimentation. Fine sediments added when streambed gravels are stable will deposit and intrude into initially clean gravels. If the fine particles are large enough to bridge openings between gravel particles, a seal will form. Then fines will deposit above this seal and fill the upper layers of the gravels. If the fine particles are small enough, they will fill the interstices of the gravels from the bottom up. As long as the gravels remain stable and do not move, adding fines to a stream can only result in the intrusion of fines or perhaps a blanketing of gravel substrates. Biologically, the presence of such conditions will generally have undesirable impacts.

If only intrusion can occur during periods when streambeds are stable, then the question may be posed, How and when are fines flushed in small streams? Apparently, flushing can occur only during periods of relatively high flows that disturb the channel bed and cause bedload transport. Field measurements we have made in Oregon's Coast Range streams indicate that the general transport of bed material (sand size and larger) occurs after discharges exceed ~0.15 $m^3 \cdot s^{-1} \cdot km^{-2}$. Based on a frequency analysis of daily streamflow values, flows exceed this level on an average of about 20 d each year. If the amount of fines is increased during high flow periods as a result of land use activities, stream energy may be used to transport and intrude the additional sediment load instead of flushing fines from the gravels. Thus, additional amounts of fines from accelerated surface or hillslope erosion during high flow events may directly influence gravel quality. Consequently, land managers must continue their efforts to control activities that have a high potential to increase stream sedimentation. Increased amounts of fine sediments must be minimized if the natural quality of a stream system is to be maintained.

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Spectral and Biochemical Characteristics of the Particulate Matter in Bedford Basin[1]

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MAYZAUD, P., AND S. TAGUCHI. 1979. Size spectral and biochemical characteristics of the particulate matter in Bedford Basin. J. Fish. Res. Board Can. 36: 211–218.

A survey of the distribution, nature, and biochemical composition of particulate matter (less than 153 μm diam) showed that small particles (<18 μm) made up the bulk of the particulate matter during most of the summer. Relatively large amounts of microzooplankton (tintinnids) were also recorded during the period of stable hydrographic conditions. Diatoms and dinoflagellates were abundant only in early fall prior to the fall bloom. All cell counts were transformed into parts per million on a volume basis to compare with Coulter Counter data. Cell counts on preserved samples strongly underestimated the number and volume of small particles and did not take into account the detritus. Considerations of the ATP content strongly suggested that for naturally occurring particulate matter there is not a constant ATP to carbon ratio but rather an upper and lower limit. Small particles were the main repository for protein whereas phytoplankton was the repository for carbohydrates. The high variability of both quality and quantity of particles in the neritic habitat suggests that a single chemical variable cannot describe fully the nutritive value of naturally occurring suspended matter.

Key words: particulate matter, phytoplankton, microzooplankton, flagellates, protein, carbohydrates, ATP, carbon

MAYZAUD, P., AND S. TAGUCHI. 1979. Size spectral and biochemical characteristics of the particulate matter in Bedford Basin. J. Fish. Res. Board Can. 36: 211–218.

A la suite d'un relevé de la distribution, nature et composition biochimique de la matière particulaire (diamètre <153 μm), nous constatons que, durant la plus grande partie de l'été, les petites particules (<18 μm) forment le gros de la matière particulaire. Nous avons aussi enregistré de grandes quantités de microzooplancton (tintinnides) durant la période de conditions hydrographiques stables. Les diatomées et les dinoflagellés ne sont abondants qu'au début de l'automne, avant la fleur d'eau automnale. Afin de les rendre comparables aux données du compteur Coulter, nous avons transformé les comptages de cellules en ppm sur la base du volume. Les nombres de cellules observés sur des échantillons conservés sous-estiment de beaucoup le nombre et le volume de petites particules et ne tiennent pas compte des détritus. Un examen de la teneur en ATP suggère fortement que, pour la matière particulaire produite naturellement, il n'y a pas de rapport ATP : carbone constant, mais plutôt une limite supérieure et inférieure. C'est surtout dans les petites particules que sont entreposées les protéines, alors que les hydrates de carbone le sont dans le phytoplancton. La grande variabilité, tant qualitative que quantitative, des particules dans l'habitat néritique donne à penser qu'une variable chimique unique ne suffit pas à décrire complètement la valeur nutritive de la matière en suspension produite naturellement.

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A number of measurements such as carbon content, chlorophyll *a*, or particulate volume have been used to characterize particulate matter in the sea. Biomass of particulate matter has also been equated with its protein or carbohydrate concentration (Marshall and Orr 1962b; Nival et al. 1972; Platt and Irwin 1973). A fraction consists of living cells which can be counted microscopically or chemically evaluated on the basis of ATP levels as shown by Holm-Hansen and Booth (1966). All too frequently investigators assume that these various measurements have some constant re-

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lationship to each other and to the particulate matter that is being studied despite widely recognized qualitative differences in the living fraction and in its proportion of the total. In this study visual and electronic (Coulter Counter) determinations of the size composition of naturally occurring particles are compared and related to their biochemical composition.

Materials and Methods

Bedford Basin is a marine basin 70 m deep, with a surface area of 17 km^2, separated from the open sea by a channel 10 km long and 20 m deep. As indicated by Platt et al. (1970) offshore water regularly enters the basin at its sill and may exchange with deep basin water by sinking directly to the bottom if it is sufficiently dense; more often exchange occurs through mixing to progressively greater depths.

Water samples were taken at a central location (44°42.3′N; 63°39.2′W) according to two sampling regimes. Temperature, salinity, chlorophyll *a*, ATP, and particulate carbon were sampled during 1973, 1974 at eight depths (0, 2.5, 5, 10, 20, 30, 40, and 60 m) using 6-L Van Dorn bottles. From June 19th to October 2nd, 1974 further samples were taken with a 30-L Niskin bottle, for the other biochemical parameters were taken near the depth of the zooplankton maximum, usually 5–10 m (circles in Fig. 1). All water samples were prefiltered through Nytex (153-μm mesh size) to eliminate large zooplankters and debris. Known volumes were then filtered either on prebaked 0.8-μm silver filters (Selas Flotronics) for carbon and nitrogen analysis or on prebaked (450°C for 30 min) Whatman GF/C filters for chlorophyll *a*, phaeopigments, protein, carbohydrate, and lipid determinations. ATP samples were filtered on GF/C filters and immediately boiled in tris buffer solution for 5 min. All other filters were frozen until analysis. Samples for total particle volume determination were stored below 5°C for up to 6 h and samples for microscopical study were preserved with Lugol's solution. In situ measurements of primary production using ^{14}C followed procedures in Taguchi et al. (1975).

Particulate carbon and nitrogen were analyzed using a Hewlett-Packard F&M 185 analyzer. Chlorophyll *a* and phaeopigments were measured by fluorometry according to the method of Yentsch and Menzel (1963), as modified by Holm-Hansen et al. (1965). ATP was measured as described by Holm-Hansen and Booth (1966). Total particle volume was estimated using a model T Coulter Counter from the sum of volumes of each channel following Sheldon (1973). Total volume was also compared with that contributed by the portion of the spectra <18 μm. Seasonal survey of the particles' spectra in the Bedford Basin (Taguchi unpublished data) indicated that 16–18 μm is the best size to separate small from large particles. Volume of the phytopankton cells, microzooplankton, and fecal pellets were estimated from linear dimensions, assuming appropriate geometric shapes (Taguchi 1976a). Protein, carbohydrate, and lipid were measured according to Mayzaud and Martin (1975). Salinities were measured with an Autolab salinometer. Chemical samples were taken in triplicate and each was analyzed 3 times.

Statistical methods were taken from Snedecor and Cochran (1973).

Results

Physical Characteristics of the Water and Distribution of the Particulate Matter

Between June 5 and October 17, 1974 a thermocline, defined here as the layer with vertical temperature gradient 0.5°C·m^{-1} (Fig. 1), of variable thickness and depth developed in the surface layers. Stratification was at its maximum between July 10 and September 4, whereas mixing of the surface layers was noticeable between June 19–July 10 and August 21–September 4. Total organic carbon had a deeper distribution than the phytoplankton, measured as chlorophyll *a*, or than ATP-related carbon. Deeper than 20-m ATP levels suggested that the carbon was largely detrital, but vertical mixing caused deepening of the surface layers rich in chlorophyll *a* and ATP carbon.

Species Composition of Phytoplankton and Microzooplankton at the Depth of the Zooplankton Maximum Layer

During summer most of the zooplankton was located in or near the thermocline and did not migrate through it (D. Mackas and M. Bohrer, Dalhousie University, Dept. Oceanography, Halifax, N.S., personnal communication). In the same layer the flagellates first reached their peak around August 8; then the diatoms *Rhizosolenia delicatula* and *Skeletenoma costatum*; and the silicoflagellates *Distephanus speculum* around September 18; and last the dinoflagellates *Ceratium* spp., *Dinophysis* spp., and *Gonyaulus* spp. around October 2 (Table 1). Except during the diatom bloom between

Table 1. Volume of four major groups of phytoplankton. All figures are expressed in 10^5 μm^3.

Date	Diatoms	Flagellates	Dinoflagellates	Silicoflagellates
June 19	6.8×10^{-3}	2.0×10^{-4}	6.7×10^{-3}	0
July 10	1.3×10^{-2}	4.0×10^{-5}	5.0×10^{-2}	0
July 24	1.5×10^{-3}	2.0×10^{-4}	1.2×10^{-3}	7.0×10^{-4}
Aug. 8	9.0×10^{-3}	5.9×10^{-2}	1.8×10^{-1}	7.0×10^{-4}
Aug. 21	1.9×10^{-2}	1.7×10^{-2}	6.4×10^{-2}	7.0×10^{-4}
Sept. 4	1.1	5.7×10^{-2}	1.0×10^{-1}	1.9×10^{-3}
Sept. 18	1.4	0	2.5×10^{-1}	2.6×10^{-3}
Oct. 2	1.9×10^{-3}	8.4×10^{-3}	9.1×10^{-1}	1.3×10^{-3}

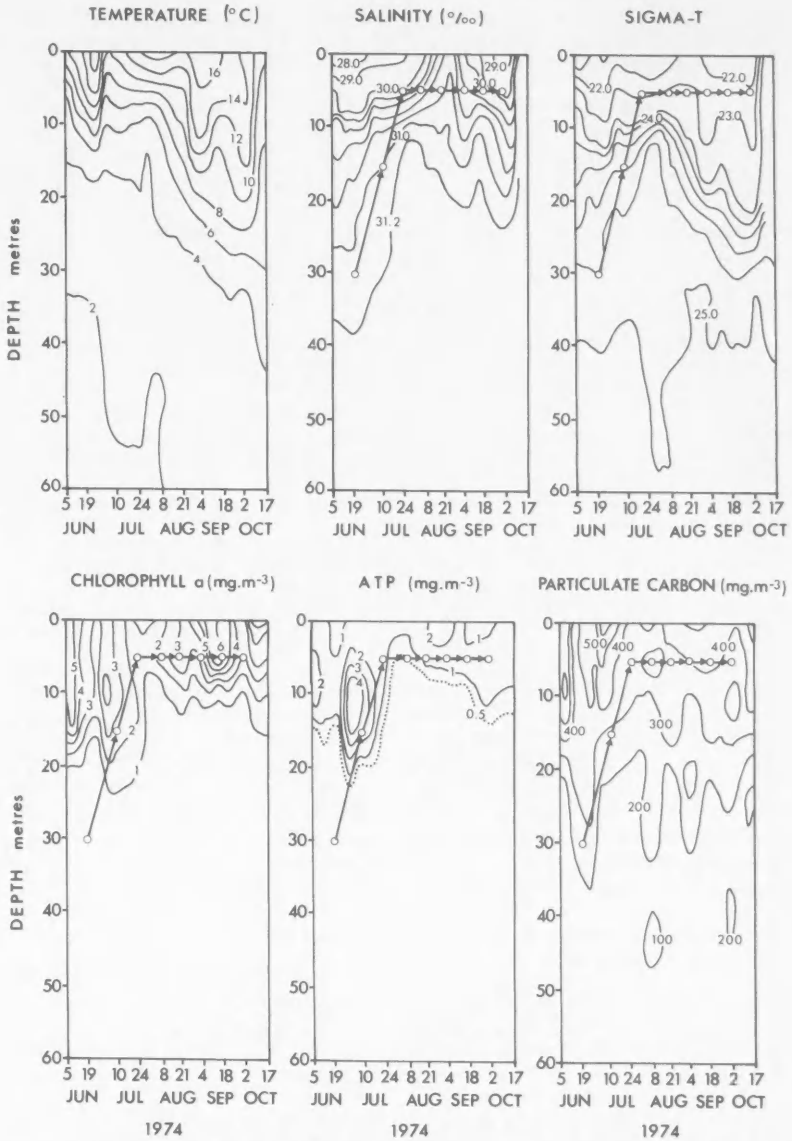

FIG. 1. Temporal distribution of temperature, salinity, σ_T, ATP, particulate carbon, and chlorophyll *a*. Circles show sampling depth.

September 4 and 19, dinoflagellates made up about 75% of the total volume of phytoplankton.

A succession of microzooplankters was also observed (Table 2). Contrary to the marked variations of the various groups of phytoplankton, the microzooplankton remained more or less constant within and/or above the thermocline, i.e. from July 24 to September 18, during which period the tintinnid *Helicostomella* constituted about 60% of the total volume.

Maximum volume of microzooplankton occurred on September 4 and maximum volume of phytoplankton on September 18 (Table 3). Microzooplankton was more important than phytoplankton in volume until the beginning of the fall bloom, during which phytoplankton made up about 25% of the total particulate matter. The size range of the major peak decreased sharply from June 19 to June 24 and then increased gradually with time; however, during the *Helicostomella*

TABLE 2. Volume of four major groups of microzooplankton and fecal pellets. All figures are expressed in 10^5 μm^3.

Date	*Helicostomella*[a]	Tintinnoinea[b]	Protozoa[c]	Copepod	Fecal pellets
June 19	0	4.6×10^{-3}	1.4×10^{-2}	0	0
July 10	9.9×10^{-2}	5.3×10^{-2}	8.7×10^{-2}	0	3.6×10^{-3}
July 24	2.8×10^{-1}	1.3×10^{-2}	2.3×10^{-2}	0	8.6×10^{-3}
Aug. 8	5.3×10^{-1}	1.4×10^{-2}	3.7×10^{-1}	0	3.7×10^{-2}
Aug. 21	2.3×10^{-1}	8.0×10^{-2}	3.0×10^{-2}	6.3×10^{-2}	5.8×10^{-2}
Sept. 4	4.3×10^{-1}	6.7×10^{-2}	4.3×10^{-2}	4.6×10^{-1}	2.2×10^{-2}
Sept. 18	4.1×10^{-1}	$12.\times10^{-1}$	1.1×10^{-1}	0	2.7×10^{-2}
Oct. 2	1.3×10^{-1}	0	8.5×10^{-2}	2.5×10^{-2}	0

[a]Also contains *Helicostomella subulata* (Ehrenberg) and *H. fusiformis* (Meunier).
[b]Also contains all species of Tintinnoinea except *Helicostomella* species.
[c]Also contains all species of Protozoa except Tintinnoinea.

TABLE 3. Volume of total phytoplankton, total microzooplankton, total particulate matter, and size range (equivalent diameter) and percentage of the major peaks of the total particulate matter. All figures are expressed in 10^5 μm^3.

Date	Phytoplankton	Microzooplankton	Total volume of particulate	Size range and % of major particles (μm)	%
June 19	1.4×10^{-2}	1.9×10^{-2}	1.5	80.6–128.0	22.1
July 10	6.3×10^{-2}	2.4×10^{-1}	5.4	25.4–128.0	72.5
July 24	9.7×10^{-2}	3.2×10^{-1}	3.9	2.0–8.0	49.7
Aug. 8	2.5×10^{-1}	9.1×10^{-1}	3.8	2.5–10.1	50.9
Aug. 21	1.0×10^{-1}	4.0×10^{-1}	4.4	2.0–25.4	68.2
Sept. 4	1.3	1.0	5.0	2.5–20.2	72.0
Sept. 18	1.6	6.5×10^{-1}	6.6	2.0–64.0	93.7
Oct. 2	9.3×10^{-1}	2.4×10^{-1}	5.5	20.2–161.0	75.9

peak (from July 24 to September 18), more than 50% of the total volume of the particulate matter was <25 μm in equivalent diameter and often < 15 μm.

CHEMICAL AND BIOCHEMICAL COMPOSITION OF THE PARTICULATE MATTER

As shown in Fig. 1, all constituents of the particulate matter decrease between July 24 and August 9. Except ATP and lipids, all the biochemical fractions followed a similar pattern of variation with a maximum on September 18 corresponding to the diatom bloom (Table 4). The ratio chlorophyll *a* to phaeopigment exceeded unity only on September 18 and October 2 which coincided with the diatom and the dinoflagellate blooms (see Table 1). The ratio of the particulate carbon to nitrogen showed unexpected variations which that if we omit the first sampling date (deep sample), probably reflected the alternation of microzooplankton-dominant periods and phytoplankton-dominant periods. Similarly, the ratio of carbohydrates to proteins was high (>0.7) when phytoplankton was abundant. Although lipids reached their maximum on September 4, there was no clear pattern of variation of the lipid to protein ratio. The ATP showed a maximum on July 10 that corresponded with a chlorophyll peak and to a lesser extent with a protein one but not with any other biochemical fraction.

Discussion

The surface layers of the Bedford Basin are relatively stable in summer. As the 24.0–24.8 isopycnal moved upward both particulate matter and zooplankton followed the shift. The 25.0 isopycnal probably indicated an intrusion of colder, denser water between July 24 and August 8. As indicated earlier, most of the suspended organic matter and the herbivorous copepods were found in or near the thermocline and did not migrate through it; thus the chemical characteristic of the particulates found at this depth is of prime importance for the trophodynamic relationships of this ecosystem.

When considering zooplankton nutrition, the volume or biomass of the particles being eaten is more meaningful than the numbers. Indeed, during the period studied the average cell volume increased from 500 μm^3 (flagellates) on August 8 to 68 000 μm^3 (dinoflagellates: *Ceratium* spp., *Dinophysis* spp., and *Gonyaulus* spp.) on October 2 (Table 1). The change

TABLE 4. Chemical composition of particulate matter with primary production. All figures are expressed in $\mu g \cdot L^{-1}$ except g $C \cdot m^{-2} \cdot d^{-1}$ for primary production. $\pm$SD = standard deviation.

Date	Chl. *a*	Phaeo	Chl. *a* / Phaeo	Carbon	Nitrogen	Carbon / Nitrogen
June 19	0.30±0.018	1.00±0.02	0.30	204±19	25±1.7	8.29
July 10	3.66±0.26	3.75±0.23	0.97	279±16	48±1.5	5.81
July 24	1.44±0.25	2.91±0.30	0.50	353±50	61±2.1	5.81
Aug. 8	2.17±0.21	5.26±0.15	0.41	279±15	45±2.0	6.29
Aug. 21	4.14±0.22	4.71±0.20	0.88	338±65	59±1.8	5.70
Sept. 4	3.53±0.19	5.79±0.30	0.61	435±36	75±7.0	5.78
Sept. 18	7.06±0.25	6.86±0.40	0.03	700±50	99±6.7	7.10
Oct. 2	3.84±0.31	2.59±0.20	1.49	414±17	60±2.0	6.93

Date	ATP	Protein	Carbohydrates	Lipids	Carbohydrate / Protein	Lipid / Protein	Primary production
June 19	0.152	185±20	84±4	38±4	0.46	0.25	1.00
July 10	3.572	392±40	172±25	47±8	0.44	0.15	1.22
July 24	1.636	346±5	196±19	44±9	0.57	0.16	0.09
Aug. 8	0.476	434±14	218±12	68±10	0.50	0.20	0.26
Aug. 21	0.612	509±20	188±3	69±7	0.37	0.17	1.10
Sept. 4	1.612	589±50	272±27	109±35	0.46	0.23	1.04
Sept. 18	1.824	604±30	453±7	92±1	0.75	0.19	0.46
Oct. 2	1.892	440±28	311±33	63±10	0.71	0.18	0.26

was consistent with the shift of the major peak in size distribution from 9.0 μm (equivalent diameter) on August 8 to 32.0 μm on October 2, accompanied by a decrease in cell numbers. The species succession of microzooplankton was less dramatic than for phytoplankton, because of the nearly continuous occurrence of *Helicostomella*, which was associated with stable hydrographic conditions (Table 2).

Serious underestimation of number and volume of preserved organisms has been reported even when Lugol's solution was used (Hulbert et al. 1960). The comparison between the measurements of total volume obtained with the Coulter Counter and by microscopic counts of preserved material confirms such an observation, phytoplankton and microzooplankton seldom accounting for more than 25% of the total volume of particles (see Table 3). Compared with the size spectra obtained with the Coulter Counter, the microscope counts (Fig. 2) do not account for particles below 10 μm diameter. According to Sheldon et al. (1973) this size category corresponds to the naked flagellates which do not preserve well. Larger particles are not underestimated by the microscopic observer to the same degree as the small ones (Fig. 2). In fact the Coulter Counter probably "sees" the tintinnids as a smaller volume than their linear dimension would suggest because the animal's body does not fill the lorica. Moreover some escape their lorica on preservation leaving the optical observer uncertain about the number of living particles at the start. Detritus particles were abundant and were probably spread across the spectrum, occasionally contributing an unexplained peak >100 μm equivalent diameter. With diatom cultures containing little detritus, good agreement is obtained between the two types of observation (Taguchi 1976b).

The proportion of detritus in the particulate carbon pool is usually evaluated by a measure of its ATP content, using the conversion factor (F = 0.4%) proposed by Holm-Hansen and Booth (1966) for estimation of cellular carbon. This method has been used with some success to estimate the proportions of detritus and living cells consummed by herbivorous copepods (Poulet 1976). Holm-Hansen (1970) claimed that this factor remains constant for most phytoplankters, although their original data (Holm-Hansen and Booth 1966) showed a rather larger variability. If bacteria and phytoplankton are considered the value of F varies between 0.05 to 0.7%. For zooplankton, values of 1–2% were reported by Blazka (1971) and Balch (1972). To see how carbon and ATP relate in naturally occurring particles we plotted ATP vs. carbon (Fig. 3) and indicated the slopes for the different values of F. Obviously, for a complex neritic system, a single factor cannot be used. Indeed some of the data apparently showed more than 100% living carbon even with a maximum F of 0.7%, in clear contradiction to the visual and Coulter count data which indicated that detritus always made up a significant portion of the medium and upper part of the spectrum. Similar observations were also reported by Sheldon et al. (1973), which they attributed to slightly different size ranges selected for the determination of particle volume and ATP. Although this variability does not rule out the use of the ATP:carbon conversion factors, it certainly

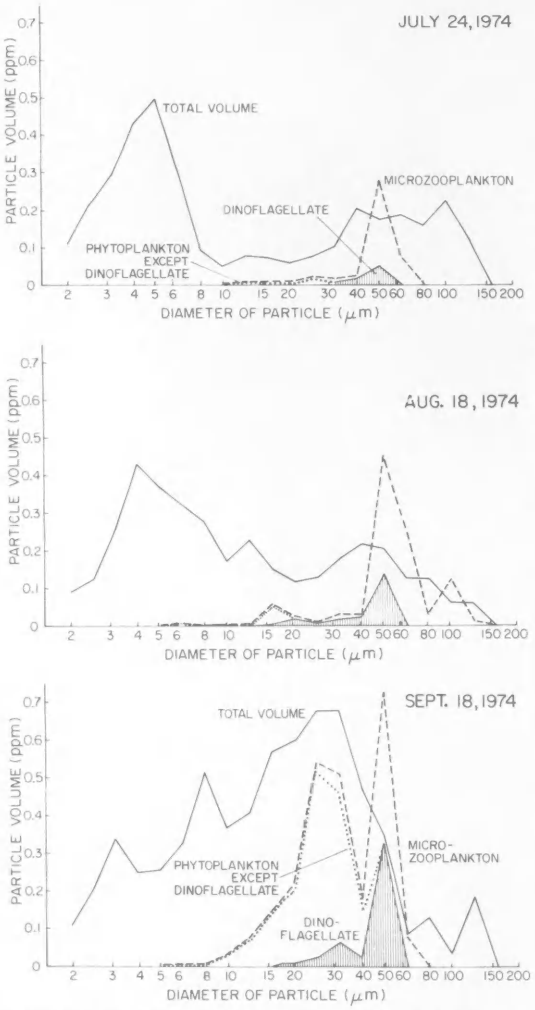

FIG. 2. Size distribution of total particles, total microzooplankton, total phytoplankton except dinoflagellates, and dinoflagellates at the depth of zooplankton-maximum layer. Solid line taken from Coulter measurement and broken lines taken from microscope measurements.

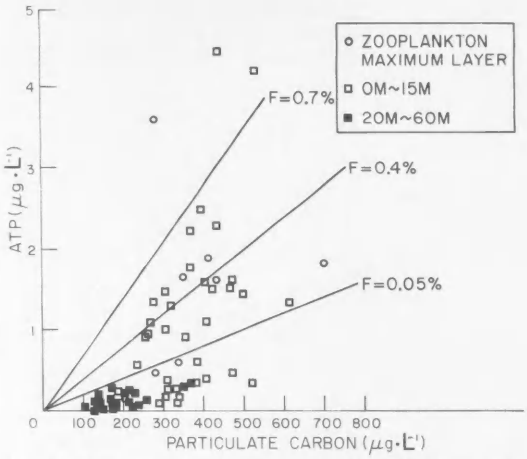

FIG. 3. Particulate carbon vs. ATP from Bedford Basin with line representing three conversion factors from ATP to living carbon.

suggests caution since serious underestimation of the living carbon can result from the use of a single F value in conditions similar to those that we observed during the summer and early fall in the Bedford Basin.

In the past, variations in the biochemical composition of phytoplankton have usually been studied with cultures. In a complex ecosystem such as the one presented here all variables are interrelated and thus most taxonomic groups are correlated with most biochemical variables. To remove the influence of the various parameters on one another, a partial correlation analysis was carried out (Table 5) which indicated the following: (1) The change in phytoplankton volume was correlated mainly with that of carbohydrates. This is consistent with the observations of Marshall and Orr (1962a) and with the fact that high ratios of carbohydrate to protein ratios followed high levels of primary production. (2) Microzooplankton volume correlated with protein and lipid. Although no data are available on the biochemical composition of microzooplankton, it probably resembles that of early stages of copepods more than that of phytoplankton. (3) The change in total volume of particles correlated with ATP and to a lesser extent with chlorophyll. (4) Small particles ($<18\ \mu m$) are mainly correlated with protein and lipids, possibly indicating that smaller phytoplankters and/or bacteria do not store large amounts of carbohydrates. Besides this biochemical variability associated with species composition, other parameters such as the stage of growth of the organisms and the water temperature can contribute to chemical variations (Antia et al. 1963; Steeman-Nielsen and Jørgensen 1968; Aaronson 1973). In any event, large cells are not the main repository of the proteins which instead are contained in the small size particle fraction. Mayzaud and Conover (1976) reached similar conclusions when studying the relationships between the digestive enzyme activities of neritic copepods and the biochemical composition of the particulate matter.

The different variables studied here could be classified in two broad categories: (1) Those such as carbon, nitrogen, and Coulter counts that estimate the total biomass irrespective of its nature and (2) those that may only consider part of the overall pool of particles (chlorophyll, carbohydrates, ATP, proteins). Because of the complexity of the neritic ecosystem, the use of

TABLE 5. Partial correlation analysis between taxonomic and biochemical parameters. Symbols: Total vol. = total volume of particles; <18 μm = volume of particles <18 μm equivalent diam; phyto vol. = volume of total phytoplankton; micro vol. = volume of total microzooplankton; diatom vol. = volume of diatoms; carbon = concentration of particulate organic carbon; nitrogen = concentration of particulate organic nitrogen; ATP = concentration of adenosine triphosphate; Chl. *a* = concentration of chlorophyll *a*; prot. = concentration of protein; carboh. = concentration of carbohydrate. *Significant at 5% confidence level; **significant at 1% confidence level; — not significant.

	Phyto.	Phyto. –Carboh.	Phyto. –Lipid	Phyto. –Chl. *a*	
Protein	—	—	—	—	
Carbohydrate	0.750*	—	0.910**	0.780*	
Lipid	—	—	—	0.700	
Chlorophyll *a*	—	—	—	—	
	Microzoo. –Prot.	**Microzoo. –Carboh.**	**Microzoo. –Lipid**	**Microzoo. –ATP**	
Protein	—	0.840**	—	0.960**	
Carbohydrate	−0.670*	—	—	0.675*	
Lipid	—	0.790*	—	0.921**	
	<18 μm Prot.	**<18 μm Carboh.**	**<18 μm Chl. *a***	**<18 μm ATP**	
Protein	—	0.826**	0.846**	0.922**	
Carbohydrate	−0.892**	—	—	—	
Lipid	—	0.761*	0.776*	0.863**	
Chlorophyll *a*	—	—	—	0.670*	
	Total vol. Prot.	**Total vol. Carboh.**	**Total vol. Lipid**	**Total vol. Chl. *a***	**Total vol. ATP**
Protein	—	—	0.950**	—	—
Carbohydrate	—	—	0.770*	—	0.920**
Lipid	−0.920**	—	—	—	0.690*
Chlorophyll *a*	0.740*	—	0.880*	—	0.930*
ATP	0.850**	0.810**	0.780*	0.610*	—

a single chemical parameter to characterize the suspended organic matter would be valid only if its taxonomical composition remains constant in time. Our data shows that this is not the case in situ and a recent study by Venrick et al. (1977) showed that it is not even the case in vitro over 24-h incubation.

Even when the quantity of the particulate matter is similar, the quality may differ. For example, though chlorophyll is greater in the fall, it is attributable mainly to large particles such as *Ceratium* that, as indicated by Conover and Mayzaud (1976), are not used to any large extent by the neritic copepods present at that time. Similarly, midsummer is characterized by relatively large amounts of microzooplankton such as *Helicostomella* whose large volume per particle (about 80 000 μm^3) might be too great for small grazers such as *Acartia* or *Eurytemora*. Tintinnids are ingested by larger species such as *Centropages* and *Temora* (Conover personal communication) and it would be interesting to investigate whether other species can do the same.

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Control of Rancidity in Frozen Pacific Herring, *Clupea harengus pallasi*: Use of Sodium Erythorbate

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BILINSKI, E., R. E. E. JONAS, AND Y. C. LAU. 1979. Control of rancidity in frozen Pacific herring, *Clupea harengus pallasi*: use of sodium erythorbate. J. Fish. Res. Board Can. 36: 219–222.

The effectiveness of 1% sodium erythorbate (NaER) and other treatments for retarding the development of rancidity was studied in Pacific herring, *Clupea harengus pallasi*, fillets stored at −28°C. Dipping of fillets in NaER significantly decreased fat oxidation. Glazing with NaER was more effective than a water glaze alone. The addition of NaER to water used for enclosing fillets in ice blocks increased the protection against rancidity. All tested NaER applications were less effective in retarding rancidity than the vacuum packaging. Erythorbic acid uptake by the flesh reaching 0.2% caused a deterioration of the flavor, whereas uptake of 0.05% or less led to no discernible alterations in flavor or appearance. Therefore, when used at low levels (uptake $\leq 0.05\%$), NaER has a potential value for extending the storage life of frozen herring.

Key words: Pacific herring, *Clupea harengus pallasi*, control of rancidity, sodium erythorbate, frozen storage

BILINSKI, E., R. E. E. JONAS, AND Y. C. LAU. 1979. Control of rancidity in frozen Pacific herring, *Clupea harengus pallasi*: use of sodium erythorbate. J. Fish. Res. Board Can. 36: 219–222.

Les auteurs étudient l'efficacité de l'érythorbate de sodium (NaER) à 1% et autres traitements visant à retarder le développement de la rancidité dans des filets de hareng du Pacifique, *Clupea harengus pallasi*, entreposés à −28°C. Le fait de tremper les filets dans le NaER réduit de façon significative l'oxydation des lipides. Le glaçage avec du NaER est plus efficace que le glaçage avec de l'eau seule. L'addition de NaER à l'eau utilisée pour enrober les filets dans des blocs de glace les protège plus longtemps contre la rancidité. Toutes les applications de NaER testées sont moins efficaces à retarder la rancidité que l'emballage sous vide. Une absorption d'acide érythorbique par les chairs atteignant 0,2% entraîne une détérioration de la saveur; par ailleurs, une absorption de 0,5% ou moins n'altère pas de façon appréciable la saveur ou l'apparence. C'est pourquoi le NaER utilisé à de bas niveaux (absorption $\leq 0{,}05\%$) peut prolonger la durée d'entreposage du hareng congelé.

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RANCIDITY is the primary cause for the deterioration of the quality of herring during frozen storage (Bilinski et al. 1978 and references cited therein). Under industrial conditions the common practice for retarding the development of rancidity in frozen herring is to glaze or encase fillets or whole fish in blocks of ice and to use low storage temperatures (−20 to −30°C). In relation to this, the use of protective agents highly soluble in water such as ascorbic acid, is of particular interest. The positive effect of ascorbic acid in retarding rancidity in fish was demonstrated by Tarr in studies involving different species of Pacific salmon (Tarr 1947, 1948). Subsequently, ascorbic acid and its stereoisomer erythorbic acid, were applied to various fishery products. For herring, ascorbic acid was tested with varying success (Konokotin 1948; Banks 1952; Marcuse 1956; Perepletchik and Novikova 1958; Anderson and Danielson 1961; Shishkanova et al. 1963; Liljemark 1964; Voss and Munkner 1966). The purpose of this investigation was to determine the usefulness of sodium erythorbate (NaER) for retarding rancidity in Pacific herring. The long-term effects of various treatments were compared in fillets stored at −28°C.

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TABLE 1. Effects of various treatments on the development of rancidity in Pacific herring, *Clupea harengus pallasi*, fillets after 8-mo storage at −28°C.

Treatment of fillets[a]	Peroxide value — milliequivalents peroxide per kg fat Mean ± SE (*n*)	Erythorbic acid content of the flesh — percent Mean ± SE (*n*)
No treatment	67.26±9.25 (6)	
Vacuum packed (Cryovac) before freezing	1.19±0.08 (6)	
Vacuum packed (Cryovac) after freezing	2.78±0.26 (6)	
Gazed with water	24.10±1.83 (6)	
Glazed with water saturated with N_2	26.05±5.05 (6)	
Glazed with 1% sodium erythorbate	5.89±1.78 (6)	0.026±0.003 (6)
Dipped in 1% sodium erythorbate at 4°C		
1 min	9.05±1.68 (6)	0.030±0.003 (4)
2 min	10.77±2.67 (6)	0.035±0.003 (4)
5 min	9.09±0.93 (6)	0.043±0.004 (4)
10 min	6.56±1.22 (6)	0.050±0.005 (4)
Dipped in water saturated in N_2 30 min at 4°C	50.18±8.26 (6)	

[a]Pacific herring caught by midwater trawl at the end of September, off SW Vancouver Island; stowed for 2 d in RSW before filleting. Average weight 178 g, fat content of the flesh 17.04 ± 1.31% (SE), $n = 6$.

TABLE 2. Comparison of various methods of chill stowage and control of rancidity in frozen Pacific herring, *C. harengus pallasi*, fillets.

Treatment of whole fish before filleting	Peroxide value (milliequivalents peroxide/kg fat) Mean ± SE (*n*)		
Experiment 1[a] (5-mo storage at −28°C)	Exposed to air	Dipped in 1% sodium erythorbate, 4°C, 5 min	Vacuum packed (Cryovac)
1.5 d in ice	19.84±2.08 (5)	6.22±1.74 (5)	1.99±0.21 (5)
1.5 d in half strength RSW	—	7.14±1.06 (6)	3.28±0.30 (6)
1.5 d in RSW	23.30±2.46 (6)	7.86±1.46 (6)	2.59±0.22 (5)
3.5 d in ice	28.59±7.06 (6)	7.18±1.37 (6)	3.91±0.10 (6)
3.5 d in half strength RSW	—	7.32±0.89 (6)	2.89±0.24 (6)
3.5 d in RSW	48.89±5.16 (6)	6.72±0.79 (6)	5.18±0.51 (6)
Experiment 2[b] (8-mo storage at −28°C)	Frozen in blocks of ice	Frozen in blocks of ice containing 1% sodium erythorbate[c]	
0 d	3.73±0.73 (9)	1.54±0.24 (6)	
2 d in ice	5.68±0.75 (8)	1.62±0.17 (6)	
2 d in RSW	12.31±2.26 (6)	3.50±1.41 (6)	
4 d in ice	9.20±3.04 (6)	4.66±1.24 (6)	
4 d in RSW	24.24±2.96 (10)	4.91±0.53 (6)	

[a]Pacific herring caught by midwater trawl in mid-November, Swanson Channel, Gulf Islands, B.C. Average weight 140 g, fat content of the flesh is 15.33 ± 1.21% (SE). $n = 6$.

[b]Pacific herring caught by midwater trawl in early November, off SW Vancouver Island. Average weight 131 g, fat content of the flesh 11.46 ± 0.91% (SE), $n = 12$.

[c]Erythorbic acid content of the flesh 0.188 ± 0.010% (SE), $n = 9$.

Materials and methods — Tests were carried out with three different lots of Pacific herring, described in footnotes in Table 1 and 2. The techniques employed for stowage of whole herring in ice and refrigerated seawater (RSW), filleting, vacuum packaging, and frozen storage were the same as those used in previously reported experiments (Bilinski et al. 1977, 1978). Food grade NaER, manufactured by Merck, Sharp and Dohme, Canada Ltd. under the trade name Neo-cebatate, was used without further purification. The dipping of fillets was conducted under conditions described in Tables 1 and 2 using unfrozen material. For glazing, previously frozen fillets were used. After dipping or glazing, the fillets were kept in air-permeable polyethylene bags in order to retard dehydration during frozen storage. In the case of fillets encased in ice blocks, with or without added 1% NaER, fillets were immersed completely in the solution, contained in polyethylene bags, and frozen in a plate freezer. The time required for lowering the temperature to −28°C amounted to 3 h.

The peroxide value (PV) and the total lipids were deter-

mined by the methods already described (Bilinski et al. 1978). The erythorbic acid content of herring flesh was measured by the 2,6-dichloroindophenol method (AOAC 1970). The results were compared statistically using Student's *t*-test.

Results and discussion — In the experiment reported in Table 1, herring fillets were subjected to various treatments aimed at retarding rancidity and PV was determined after 8-mo storage at −28°C. In the control fillets, which received no treatment and remained exposed to air during storage, the rancid spoilage was already very advanced. In fillets vacuum packed before freezing, PV was significantly lower ($P < 0.001$) than in fillets vacuum packed after freezing, but both treatments provided very good protection. A thorough glazing of fillets with water inhibited to some extent the oxidative deterioration; however, after 8 mo at −28°C the rancidity had already reached comparatively high levels. Glazing with water saturated with N_2 gave no additional protection. A glaze containing 1% NaER was much more effective than the water glaze, ($P < 0.001$). Dipping of fillets in 1% NaER also reduced the rancidity to a large degree. There was no statistically significant difference in PV between fillets dipped in NaER for 1–5 min. Prolonged dipping of fillets in water saturated with N_2 had no effect on the development of rancidity.

In two experiments (Table 2) the whole herring was kept under different conditions of chill stowage before filleting and freezing. In the first experiment, fillets exposed to air or dipped in NaER or vacuum packed were compared after 5 mo at −28°C. In fillets dipped in NaER, PV was at least 3 times lower than in air-exposed fillets, ($P < 0.001$), but it was significantly higher than in vacuum-packed fillets, ($P < 0.005$). The method or duration of chill stowage had no significant effect on PV in NaER treated fillets.

In the second experiment (Table 2) fillets were encased in blocks of ice, with or without added 1% NaER, and rancidity was measured after 8-mo storage at −28°C. In fillets frozen in water the chill stowage accelerated the development of rancidity and this effect was more pronounced in RSW than in ice. With one exception (4 d in ice), the PV was significantly lower ($P = 0.02$–0.001) when fillets were frozen in 1% NaER rather than in water.

The erythorbic acid content of the flesh was determined in fillets treated with NaER. The lowest levels were found in fillets with glaze containing NaER. In fillets dipped for up to 10 min in 1% NaER the erythorbic acid content of the flesh amounted to 0.05% or less. In fillets frozen in a solution of 1% NaER the uptake of erythorbic acid of the flesh was much higher and approached 0.2%. In the latter case the uptake of erythorbic acid must be reduced by using lower NaER concentrations and/or faster freezing rates.

A sensory evaluation of raw and cooked fillets was made to detect the possible side effects of NaER treatments on the appearance and flavor of the flesh. After thawing, fillets containing 0.2% erythorbic acid had a slightly more pronounced pink shade than the vacuum-packed fillets, but after cooking (10 min steaming) there was no apparent difference. However, the cooked flesh could be distinguished by a changed flavor, which appears to be due to a masking of the characteristic, somewhat sweet taste of untreated herring flesh. On the other hand, fillets that absorbed 0.05% or less erythorbic acid showed no discernible alterations in flavor or appearance of the flesh.

The results of the present study indicate that NaER is effective in retarding the development of rancidity in frozen herring fillets and that it increases the protection provided by glazing or freezing fillets in ice blocks. Under conditions tested, the treatment of fillets with NaER gave less protection than vacuum packaging, but it is more readily applicable in conjunction with the existing industrial practice of glazing or encasing herring in ice. The use of NaER seems particularly advisable for herring stowed in RSW before freezing. The chill stowage of herring in RSW promotes the development of rancidity (Bilinski et al. 1978) but this effect is largely suppressed in fillets treated with NaER. In earlier work, Liljemark (1964) found that dipping of herring fillets in ascorbic acid or vacuum packaging improved the quality retention about as much as a temperature reduction from −20 to −30°C. In some studies it was reported that ascorbic acid has no practical value for controlling the rancidity in herring because it has a pronounced adverse effect on the flavor or the appearance (Banks 1952; Voss and Munkner 1966). However, the levels of ascorbic acid in the flesh, associated with these changes, were not determined in these studies. Our results indicate that the uptake of 0.03–0.05% erythorbic acid provides a significant protection against rancidity without altering to a discernible degree the flavor or appearance of herring flesh. In earlier studies with other species of fish, it was found that the uptake of 0.05% ascorbic acid retarded the development of rancidity (Tarr 1947; Bauernfeind et al. 1951). In conclusion, it is suggested that NaER used at low levels, preferably in combination with glazing and similar treatments for protecting fish from a direct contact with air, has a potential value for extending the storage life of frozen herring.

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Immersion Vaccination of Sockeye Salmon (*Oncorhynchus nerka*) with Two Pathogenic Strains of *Vibrio anguillarum*

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GOULD, R. W., R. ANTIPA, AND D. F. AMEND. 1979. Immersion vaccination of sockeye salmon (*Oncorhynchus nerka*) with two pathogenic strains of *Vibrio anguillarum*. J. Fish. Res. Board Can. 36: 222–225.

Sockeye salmon (*Oncorhynchus nerka*) were immersion-vaccinated in suspensions containing 5×10^7, 5×10^6, 5×10^5, or 5×10^4 bacteria/mL of bivalent or monovalent, formalin-killed *Vibrio anguillarum*, Types I and II. The fish were split into two lots and held for 54 d. At that time one lot was challenged with living, virulent *V. anguillarum*, Type I, and one with living, virulent *V. anguillarum*, Type II. Immunization with bivalent bacterin effectively protected the fish from vibriosis, but monovalent vaccine was effective only against the homologous challenge. Immunization with the highest concentration of Type I monovalent bacterin resulted in 0% Type I and 58% Type II challenge mortality. Immunization with the highest concentration of Type II monovalent bacterin resulted in 41% Type I and 0% Type II challenge mortality. Immunization with the highest concentration of bivalent Type I/Type II bacterin resulted in 2% mortality in both challenges. Protective bacterins were effective at concentrations down to 5×10^5 bacteria/mL.

Key words: immersion vaccination, bivalent vaccines, *Vibrio anguillarum*, vibriosis

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Nous avons vacciné des saumons nerka (*Oncorhynchus nerka*) par immersion dans des suspensions contenant 5×10^7, 5×10^6, 5×10^5, ou 5×10^4 bactéries/mL de *Vibrio anguillarum* des types I et II, bivalents ou monovalents, tués à la formaline. Nous avons

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séparé les poissons en deux lots et les avons gardés ainsi durant 54 jours. Un lot a alors été exposé à *V. anguillarum* virulent et vivant du type I, et l'autre à *V. anguillarum* virulent et vivant du type II. L'immunisation par la bactérine bivalente protège effectivement les poissons contre la vibriose, alors que le vaccin monovalent n'est efficace que contre l'attaque de son homologue. L'immunisation par la plus forte concentration de bactérine monovalente du type I résulte en des mortalités de 0% avec le type I et de 58% avec le type II. L'immunisation par la plus forte concentration de bactérine monovalente du type II résulte en des mortalités de 41% avec le type I et de 0% avec le type II. L'immunisation par la plus grande concentration de bactérine bivalente du type I/type II cause des mortalités de 2% dans les deux attaques. Les bactérines protectrices sont efficaces à des concentrations aussi basses que 5×10^5 bactéries/mL.

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VIBRIOSIS, caused by *Vibrio anguillarum* is responsible for massive mortality of saltwater-reared salmonids and other marine fishes (Smith 1961; Anderson and Conroy 1970; Fryer et al. 1972). Vaccination is gaining popularity among aquaculturists as a prophylactic control method. Evidence indicates that vaccines against vibriosis are effective and economical (Fryer et al. 1972; Rohovec et al. 1975; Antipa 1976; Antipa and Amend 1977; Croy and Amend 1977; Schiewe and Hodgins 1977). Presently there are two major serotypes of *V. anguillarum* causing disease in salmonids. The first serotype, Type I ("fast growing", 775 type), is widely distributed and well characterized (Smith 1961; Anderson and Conroy 1970; Evelyn 1971; Fryer et al. 1972). The second serotype, Type II ("slow growing" 1669 type), has only recently been characterized (Harrell et al. 1976; Schiewe and Hodgins 1977) but has been isolated from fish in North America, Europe, and Japan (unpublished observations on cultures isolated throughout these three areas). The recognition of these serotypes, as well as published accounts of less widespread serotypes (Sawyer and Strout 1977), indicates the possible need for a multivalent bacterin preparation.

Several techniques are now available for mass immunization of salmonids. These include injection, oral delivery, hyperosmotic infiltration (a pre-dip in high osmolarity solution followed by a dip in vaccine), spray vaccination, and immersion (Corbel 1975; Amend 1976; Amend and Fender 1976; Schachte 1976; Antipa and Amend 1977; Croy and Amend 1977; Fryer et al. 1977; Gould 1977). Each method, except injection, delivers unquantitated vaccine doses to elicit an as yet uncharacterized immune response in fishes. Because of the unknown character of mechanisms providing immunity, each delivery technique requires evaluation with each vaccine type. The purpose of this investigation was to determine efficacy, serotype cross-protection, and presence or absence of antigenic competition with immersion-delivered, bivalent *V. anguillarum* bacterin.

Materials and methods — *Vibrio anguillarum*, serotypes I and II, were obtained from M. Scheiwe (National Marine Fisheries Service, Seattle, Washington). Separate, formalin-killed bacterins of each serotype were prepared according to Croy and Amend (1977). A stock solution of bivalent bacterin was prepared by combining equal parts of each monovalent bacterin previously standardized to 1×10^9 organisms/mL in sterile 0.85% saline. The bivalent bacterin thus contained 5×10^8 bacteria/mL of each serotype. Before immunization stock solutions of monovalent bacterin were prepared by further diluting the 1×10^9 bacteria/mL suspensions to 5×10^8 bacteria/mL with sterile 0.85% saline.

To vaccinate fish, Type I, Type II, and bivalent stock bacterins were each diluted tenfold, from 10^{-1} to 10^{-4}, in 1400 mL volumes of 0.85% saline. Sockeye salmon (*Oncorhynchus nerka*) weighing 8 g each (raised from eggs in vibriosis-free water at TAVOLEK research facility) were vaccinated by immersing approximately 80 fish in each dilution for 2 min. The final immersion ratio was 454 g of fish/L of bacterin. Two control groups were sham vaccinated in 0.85% saline.

All groups of fish were split into two lots and held for 54 d at a mean temperature of 10°C and then challenged by the waterborne route. One lot was challenged with Type I and one with Type II, living, virulent *V. anguillarum*. Each challenge solution consisted of 24-h culture diluted 10^{-3} in 4 L of 0.85% saline. The fish were exposed for 17 min at 17°C (except the three groups indicated in Table 1 which because of a timing error were challenged 10 min). The Type I challenge contained 4.9×10^9 viable organisms/mL and the Type II challenge 2.1×10^9 viable organisms/mL. The fish were held at 17°C for 8 d. The challenge period was terminated when death had essentially ceased in all lots.

All dead fish were examined to determine the etiological agent. Samples from the kidney were inoculated on Trypticase® Soy Agar plates for preliminary bacterial isolation. Colony morphology, gram stain, slide agglutination, and sensitivity to vibriostat 0/129 were used to identify the organism.

Results and discussion — Sockeye salmon immunized with Type II or bivalent bacterin were protected from a Type II challenge, but immunization with Type I resulted in no significant protection against Type II challenge (Table 1). The bivalent bacterin was as effective as Type II bacterin in protecting the fish from Type II vibriosis. Dilutions of 10^{-1} and 10^{-2} showed almost complete protection, while 10^{-3} and 10^{-4} dilutions were progressively less protective.

Challenge with Type I resulted in high losses in the

TABLE 1. Cumulative mortality of sockeye salmon challenged 54 d postimmunization with 4.9×10^6 organisms/mL Type I or 2.1×10^6 organisms/mL Type II *Vibrio anguillarum*.

Bacterin dilution $\log_{10}$	Losses during the prechallenge period		No. of fish challenged		No. died of vibriosis		Mortality (%)	
	Type I	Type II	Type I	Type II	Type I	Type II	Type I	Type II
Challenged (Control)[a]	8	2	32	32	6	16	19[b]	50
Unchallenged (Control)	16	16	24	21	0	0	0	0
Type I/Type II (bivalent)								
10^{-1}	1	4	39	36	1	1	2	2
10^{-2}	0	3	40	38	0	0	0	0
10^{-3}[a]	1	4	39	35	1	1	2	2
10^{-4}[a]	0	8	40	31	8	6	20	19
Type I (monovalent)								
10^{-1}	0	0	40	39	0	23	0	58
10^{-2}	0	18	40	21	0	11	0	52
10^{-3}	0	3	40	36	3	24	7	66
10^{-4}	0	7	40	33	4	14	10	42
Type II (monovalent)								
10^{-1}	0	0	39	38	16	0	41[b]	0
10^{-2}	1	0	40	35	18	1	45[b]	2
10^{-3}	0	1	40	36	23	3	57[b]	8
10^{-4}	0	0	40	38	10	10	25[b]	26

[a]In the Type I challenge only, these groups were exposed for 10 min.

[b]No comparison of mortality between the Type I challenged control fish and the Type I challenged monovalent Type II immunized fish is made because of the different challenge exposures (see footnote a).

Type II vaccinates, while the Type I and bivalent groups were protected (Table 1). Again, there was no evidence of antigenic interference. Protection from the Type I preparation was reduced at the 10^{-3} and 10^{-4} dilutions. There was no evidence of antigenic interference.

There is an indication in the data that immunization with monovalent bacterin results in increased susceptibility to the hetrologous challenge. Unfortunately, reduced exposure time for the Type I control group challenge resulted in less experimental evidence for such a conclusion (Table 1). There is, however, reduced heterologous challenge mortality in groups treated with monovalent bacterin at the highest immunizing dilutions. Additional experimentation is required to better understand this phenomenon.

Nitrogen supersaturation in the water supply caused high prechallenge losses in some test groups. The degassing system on the water inflow to certain tanks was defective. The prechallenge losses do not appear to be associated with any particular treatment.

According to Schiewe and Hodgins (1977) and Gould (1977) both serotypes were, to a degree, cross-protective when injected in large doses. A higher degree of protection occurred when bacterins were homologous to the challenge organism. With immersion delivery used in this investigation, the lack of protection against hetrologous challenge indicates the need for bivalent bacterin in areas endemic for both strains.

Antigenic competition in animals has been observed with the administration of two or more antigens. This phenomenon often depends on the antigen formulation, location, or method of injection (Pross and Eidenger 1974). Schiewe and Hodgins (1977) found that 2-mg doses of bivalent bacterin (1:1 wt/wt), intraperitoneally injected, effectively immunized against challenge by both strains. Antigenic competition was not observed. Comparing all dilutions of monovalent vs. bivalent bacterin delivered by immersion, antigenic competition was again not observed. A single exposure diluted as much as 10^{-2} was efficacious without significant loss of potency. Thus, immersion of fish in bivalent bacterin is an effective means of immunization.

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Expressing the Phosphorus Loading Concept in Probabilistic Terms[1]

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CHAPRA, S. C., AND K. H. RECKHOW. 1979. Expressing the phosphorus loading concept in probabilistic terms. J. Fish. Res. Board Can. 36: 225–229.

Data from North Temperate lakes were used to estimate the uncertainty of a phosphorus loading model. A number of graphical representations of the uncertainty were developed to demonstrate the importance of including probabilistic information in phosphorus loading calculations.

Key words: limnology, lake management, phosphorus, eutrophication, models, statistics, probability, loading, lakes, prediction

CHAPRA, S. C., AND K. H. RECKHOW. 1979. Expressing the phosphorus loading concept in probabilistic terms. J. Fish. Res. Board Can. 36: 225–229.

Les auteurs ont utilisé des données provenant de lacs tempérés septentrionaux pour estimer l'incertitude d'un modèle de charge de phosphore. Ils donnent plusieurs représentations graphiques de l'incertitude afin de démontrer l'importance qu'il y a à inclure les renseignements probabilistes dans les calculs de la charge de phosphore.

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TABLE 1. Means and extremes of the variables used for model development.

Variable	Symbol	Units	Minimum	Geometric mean	Maximum
Mean depth	z	m	0.73	7.2	88.7
Water residence time	τ_w	yr	0.003	0.40	33.7
Influent phosphorus concentration	$[P]_I$	mg/m^3	7.9	50.3	450
Lake phosphorus concentration	$[P]_\lambda$	mg/m^3	4.0	24.1	209

VOLLENWEIDER's (1968, 1975, 1976) phosphorus loading plots can be used to predict a lake's trophic state on the basis of variables representing its phosphorus loading, morphometry, and hydrology. Although such plots are statistical in the sense that they represent generalizations drawn from large numbers of lakes, they are deterministic in that a fixed predicted value results from any given set of independent variables. Such a prediction is a useful estimate of the most likely outcome, but does not include information on the reliability or uncertainty of the prediction. Recently, Vollenweider (personal communication) suggested that additional research would be required to improve significantly on lake trophic state calculations. In essence, this is a recognition that existing models have been developed to the limits of their ability to explain the variability of available data. The primary purpose of the present paper will be to quantify the unexplained variability, or uncertainty of a phosphorus loading model, and to demonstrate how such probabilistic information can be included in management applications. This is done by using statistical methods to estimate the error in the predictions of Vollenweider's (1976) most recent phosphorus loading criteria model.

Quantification of prediction uncertainty is a relatively recent addition to the literature on the development and application of lake models. Reckhow (1977) and Walker (1977) developed black-box lake models and phosphorus loading criteria that include an analysis of uncertainty and expressions for a probabilistic assessment of lake trophic state classification. Reckhow (1978a, 1979) has discussed how consideration of uncertainty can be helpful in nutrient budget sampling design and in the evaluation of lake management strategies. For instance, prediction uncertainty expresses the inverse of the value of the information contained in a prediction, and thus should be considered as important as the expected, or most likely, value. When provided with a value for prediction uncertainty, the user is better able to evaluate and utilize the information provided by a model. For that reason, techniques are being proposed here and elsewhere (Reckhow 1978b) for including prediction uncertainty in the model application process.

Data — The data represent 117 North Temperate lakes compiled from Vollenweider (1968), Dillon and Rigler (1974), National Eutrophication Survey (1975), Rast and Lee (1978), and W. C. Sonzogni (Great Lakes Basin Commission, Ann Arbor, Mich., personal communication). The general characteristics of these lakes are summarized in Table 1. Using criteria (Table 2) based on in-lake total phosphorus concentration, $[P]_\lambda$, the lakes may be further categorized by trophic state as 30 oligotrophic, 23 mesotrophic, 36 eutrophic, and 28 hypereutrophic.

The Model — The model used in the present analysis represents the state-of-the-art of phosphorus loading calculations and will be called Vollenweider (1976)'s Improved Loading Criteria Model (VILCM). VILCM is expressed mathematically as

$$[\hat{P}]_{\lambda,\mathrm{VILCM}} = \frac{[P]_I}{1 + \sqrt{\tau_w}}, \tag{1}$$

where $[\hat{P}]_{\lambda,\mathrm{VILCM}}$ is the predicted concentration of total phosphorus in the lake (milligrams per cubic metre), τ_w is the lake's measured water residence time (years), and $[P]_I$ is the measured phosphorus inflow concentration which represents the loading to the lake divided by the water flow into the lake. Coincidentally, at least three other investigators (Larsen and Mercier 1976; Reckhow 1977; and Walker 1977) have independently derived similar relationships. It should be noted that $[P]_I$ can be related to the more conventional expression of loading, L_c (milligrams per square metre per year), by

$$L_c = \frac{\bar{z}[P]_I}{\tau_w} \tag{2}$$

where $\bar{z}$ is the mean depth of the lake (metres).

Analysis — A nonlinear least-squares regression algorithm

TABLE 2. Trophic classification scheme based on in-lake total phosphorus concentration in milligrams per cubic metre for North Temperate lakes (generally based on Dillon and Rigler 1975).

Trophic state	$[P]_\lambda$ (mg·m^{-3})
Oligotrophic	<10
Mesotrophic	10–20
Eutrophic	20–50
Hypereutrophic[a]	>50

[a]Approximately equivalent to lakes greater than Dillon and Rigler's (1975) Level 4.

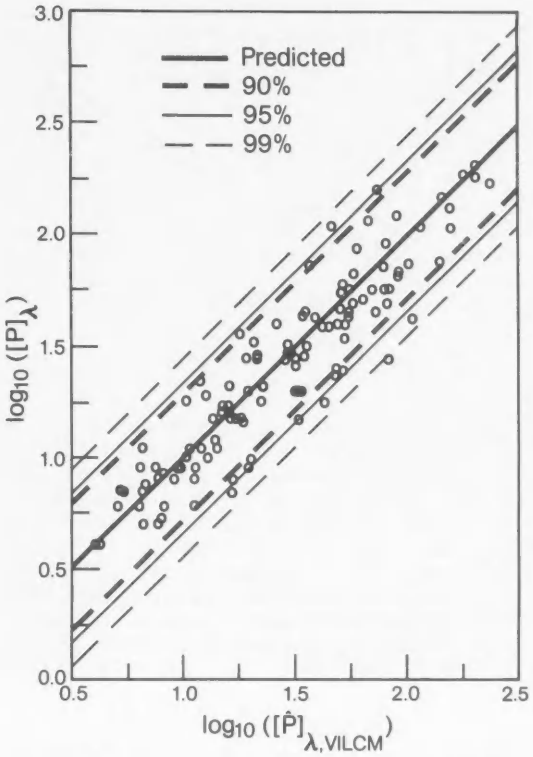

FIG. 1. Comparison of model and data on a logarithmic scale with prediction intervals.

(Marquardt 1963) was used to estimate the coefficients α and β for

$$\log [P]_\lambda = \log \frac{[P]_i}{1 + \alpha \tau_w^{\beta}}, \tag{3}$$

where $[P]_\lambda$ is the measured concentration of total phosphorus in the lake (milligrams per cubic metre). The logarithmic transformation is needed to maintain homoscedasticity. The resulting coefficient estimates were $\hat{\alpha} = 1.17$ and $\hat{\beta} = 0.45$ and the coefficient of determination, R, was 0.92. A joint confidence region constructed for $(\hat{\beta}, \hat{\alpha})$ as described in Draper and Smith (1966) was used to determine that (0.5, 1.0) cannot be rejected as the true value of (β, α) at the 5% significance level, i.e. it lies well within the (approx.) 95% confidence region contour. It was, therefore, concluded that VILCM is an adequate model of the data base.

While the uncertainty of the nonlinear regression model could have been estimated, it was considered of more practical importance to analyze the error associated with VILCM directly. To do this, we determined the residual error by directly comparing the predictions of VILCM and measurements (logarithmic transforms were employed again

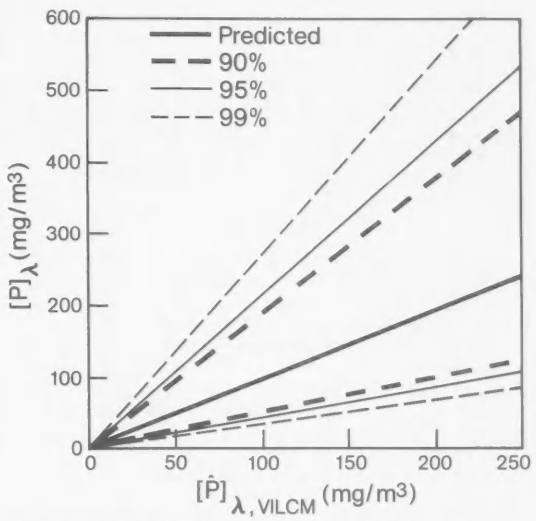

FIG. 2. Prediction intervals on an untransformed scale.

to maintain homoscedasticity). The residual variance, s^2, was then calculated as

$$s^2 = \frac{\sum(\log [P]_\lambda - \log [\hat{P}]_{\lambda,VILCM})^2}{(n-1)}, \tag{4}$$

where s^2 is assumed to be an estimate of the variance of the prediction of an individual observation using VILCM. By assuming that the error about each prediction is normally distributed, confidence intervals can be constructed for the model. The above procedure is believed to be justified because (1) for large numbers of observations the normal distribution closely approximates the Student t distribution, which is conventionally used to construct confidence intervals; (2) the error in the prediction attributable to parameter uncertainty was determined to be negligible, i.e. approximately an order of magnitude smaller than the residual standard deviation; and (3) the bias introduced by assuming that VILCM is the "true" model was determined to be negligible.

Results — Figures 1 and 2 show 90, 95, and 99% prediction intervals for transformed and untransformed scales. An alternative expression, which is closer in spirit to the original conception of a Vollenweider prediction, is shown in Fig. 3, where cumulative probability curves are used to partition the probability into trophic classes. Finally, a probabilistic loading plot is shown in Fig. 4.

The usefulness of these graphs can be demonstrated by making predictions for a lake with a measured inflow concentration of 100 mg/m^3, a mean depth of 10 m and a water residence time of 16 yr. Equation (1) is first employed to calculate a deterministic prediction of in-lake phosphorus concentration of 20 mg/m^3. From Fig. 2, we see that such a prediction has a 95% confidence interval from 9 to 42 mg/m^3. Figure 3 can also

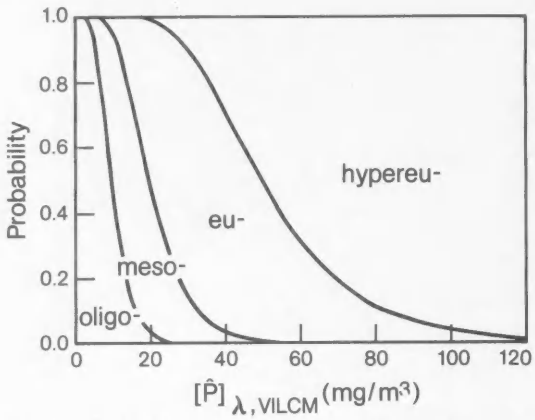

FIG. 3. Probability of a prediction falling within a particular trophic class.

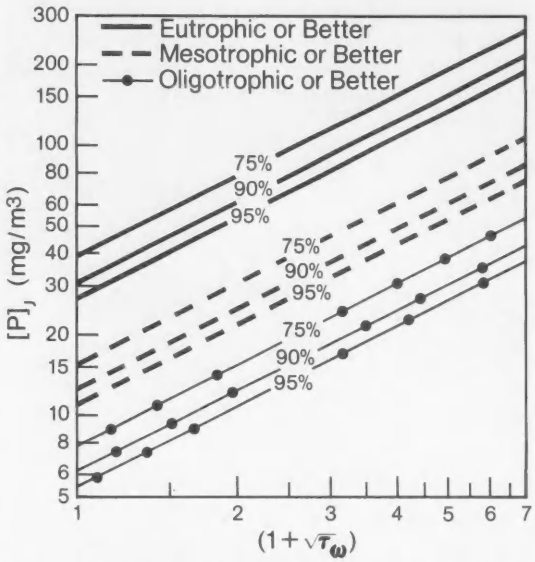

FIG. 4. Probabilistic loading plot showing the logarithm of the predicted inflow concentration as a function of the water residence time. Percentages represent the certainty of the effectiveness of the inflow concentration achieving the expected trophic state.

be used to estimate that the lake has a 4% chance of being oligotrophic (<10 mg/m³), a 46% chance of being mesotrophic (10–20 mg/m³), and a 50% chance of being eutrophic (>20 mg/m³). (There is also a chance that the lake would be hypereutrophic (>50 mg/m³), although it is negligible). This latter form of the prediction demonstrates the importance of including uncertainty in management applications of the model. Such a probabilistic expression makes it clear that lowering the in-lake concentration to levels at or below 20 mg/m³ does not necessarily ensure that the lake will be mesotrophic (<20 mg/m³). Actually, in this case the lake still has a 50% change of being eutrophic (>20 mg/m³). While this might seem obvious, there is a tendency to think of trophic class boundaries as thresholds. Hopefully, the foregoing example makes clear the probabilistic nature of trophic state calculations. It also suggests an alternative approach to loading calculations by designing the waste removal program to achieve a desired trophic state with a specified probability. For instance, it might be desired that the example lake be 95% certain of being less than eutrophic (<20 mg/m³). Figure 4 can then be used to estimate that the inflow concentration would have to be lowered to approximately 50 mg/m³ (or using equation 2 an areal loading of 31 mg/m² per yr).

Discussion — While explicit consideration of uncertainty enhances the usefulness of lake classification schemes, this procedure is not without shortcomings. Limitations on its use are

1) Like all empirical models, the results are applicable only within the range of conditions represented by the model development data set. In this case, the hydrologic, morphometric, and trophic range covers most natural lakes, but the climatic range is restricted to the North Temperate zone.

2) The model is based on the assumption of completely mixed conditions in a lake, and the model predicts the "average" (not specified temporally or spatially but typically referring to whole-lake means for the ice-free period; see references for additional information) phosphorus concentration in a lake. Thus the probabilities calculated provide estimates of the lake trophic state based on *average* conditions alone. Local conditions may deviate significantly, temporally, and/or spatially, from the average, but the model is not capable of describing these local variations.

3) There are errors in the estimates of the model variables for the model development data set. In particular, phosphorus inflow concentration, $[P]_j$, may be quite uncertain. This variable error results in an increase in the model standard error, s, and it slightly biases the model coefficients. Prediction uncertainty must include the effect of all sources of uncertainty, including the model variables. Thus, equation (4), with s already increased by variable error, is an appropriate measure of the prediction interval when data for the application lake are gathered at about the same level of uncertainty as those for the model development. Many applications of lake models, however, are based on indirect estimates of phosphorus loading (e.g. literature export coefficients). These estimates generally are much less certain than measured values, and thus an additional component for loading uncertainty (reflecting the additional loading uncertainty beyond that in the model development data set) must be added, in

variance form, to the model's residual variance.

Likewise, error in the measured phosphorus concentration in a lake (the dependent variable) is incorporated in the standard error of the model through the regression analysis. Applications of the model do not require inclusion of this dependent variable error component; therefore, the standard error presented in this paper is slightly inflated by this term.

4) Finally, while we have assumed that phosphorus enrichment is eutrophication (well nourished), it can be argued (W. W. Walker, Metasystems, Inc., Cambridge, Mass., personal communication) that parameters representing the deleterious effects of overenrichment (e.g. chlorophyll *a*, Secchi depth, or dissolved oxygen) would provide a more realistic basis for the analysis of the uncertainty of lake trophic state calculations. Nevertheless, total phosphorus concentration is often used as the basis for lake water quality criteria or standards, and in such cases the present approach would be appropriate. Additionally, it should be stressed that the present paper does not purport to represent the last word in lake uncertainty analysis but rather is intended to introduce the concept of probability to lake quality assessment. Limitations of the approach, particularly with respect to additional variable error, indicate that this discussion provides a "first-cut" at a technique that requires additional consideration and study.

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The inability to relate common names used for fish and fish products in various countries to scientific names and technical processes has been a major source of misunderstanding and confusion in the international trading of fish and fish products. The Publication by OECD in 1968 of the first edition of its multilingual dictionary represented a milestone in achieving international understanding of the terminology used in the commercial production and marketing of fish and fish products.

This second edition contains a number of new entries reflecting technological developments as well as an expansion and updating of many of the original items.

In both editions each entry is fully described in both English and French and most of the terms are translated into 13 other languages: Danish, Dutch, German, Greek, Icelandic, Italian, Japanese, Norwegian, Portuguese, Serbo-Croatian, Spanish, Swedish, and Turkish. For convenience of reference the dictionary contains an index in each of the above mentioned languages as well as an index of Scientific Names.

The contents of this dictionary are based on contributions received from individuals representing many countries involved in international trade of fish and fish products.

While the primary purpose of this book is not to promote uniformity of fish names, nomenclatures used in the "F.A.O. Yearbook of Fishery Statistics," the "ICES Bulletin Statistique des Pêches Maritimes," and the "ICNAF Statistical Bulletin" have been taken into account to reflect the attempts by these organizations towards harmonization of fish names. Differences existing between such organizations (i.e. Saithe or Coalfish in ICES; Pollock in ICNAF) have been recorded as well as many local names.

Main items are cross referenced to other entries by referring to them in capitals preceded by a "+" such as "+ CHINOOK." The use in the cross reference of the locator number as well (e.g. "+ 191 — CHINOOK") would greatly facilitate the use of this dictionary.

Items pertaining to common names of fish and fish products usually include a brief description of the commonly marketed forms (e.g. for Clams — Frozen: in shell, or shelled meats). Entries pertaining to processes such as "Salt Cured Fish" include a description of the method(s) of preparation.

Users of this book are advised that though it reflects the majority of fish and fish products and fishing industry terminology in international trade, it is not all inclusive. Variances in the usage of local common names among countries using the same language cannot all be included. This does not seriously detract from this work or its usefulness and it is strongly recommended to any user of fishing industry books, periodicals, and journals who has a need for a quick and ready reference on fish and fish product terminology. The reviewer especially recommends this dictionary to anyone involved in the import or export of fish and fish products.

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(R228)

Marine Ecology. Vol. III. Cultivation, part 2. *Edited by O. Kinne. John Wiley & Sons, Inc., New York, N.Y. 1977. xv + p. 578–1293. $82.80.*

With this publication, Kinne has reached the midpoint in his comprehensive review of marine ecology, a subject that reaches into virtually every branch of natural science. Kinne is the sole contributor to this part which is further subtitled "Cultivation of Animals — Research Cultivation." It is a section of a single chapter of the treatise. There are 14 subsections, beginning with protozoa and proceeding through the phyla to mammals. Kinne pays particular attention to the problems encountered by workers with respect to delicacy of life cycle phases, and in each section sensitivity of organisms to handling and to laboratory conditions as well as to apparatus is included. Work on organisms as used in toxicity tests and as food organisms is treated; as well, thought-provoking summaries of the work done on each phyletic group are included. It is here that Kinne indicates fruitful directions for future work to take.

Since the first outline for Marine Ecology was proposed, several changes have been made. Kinne decided to treat the axenic cultivation of animals in Volume III, part 3, but this decision creates some problems as the subject is introduced in part 2; frequent cross references are necessary. However, it is fitting for axenic culture to be the introduction to treatments of aquaculture and of microcosms which are among the subjects chosen to complete Vol. III.

There are several ways in which this work suffers. The narrative does not always flow in the main body of the text, since, at least in part, the language is that of the author whose work is being reviewed. It is at the end of each section where Kinne pulls together the threads of the research that the writing is integrated and well-controlled.

Some work receives less than complete citation. For example, to omit to highlight the contributions of G. Kidder and V. Dewey to the culture of *Tetrahymena* and at the same time to cite at some length less fundamental work seems less than just.

The frequent inclusion of pages of media formulation is part of what makes this book so long. It is doubtful that it will be used as a culture manual, and, therefore, it would have been better to discuss the results of using more and more refined media than to include the recipes wholesale.

Indeed, in one case (Base S medium for protozoa, p. 598), part of the formulation (PII metals) is missing, so reference to the original work is necessary anyway. In passing, the formulation for PII metals appears in the Crustacea section (p. 775), but no cross reference is made in the text or index.

The subject index is not very useful. There is one citation under "Food organisms, protozoa," but this subject is treated in various places in the text (e.g. p. 688, 790, 975 and 981). The taxonomic index is more complete, but since it is set up in the usual way, one must look up each reference to see why the organism is cited.

On balance, one can only admire Kinne's courage in undertaking so monumental a task. No one working in marine research should be without easy access to this treatise which forms a background against which work in marine ecology will be done for some time to come.

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(R219)

An Introduction to the Aquatic Insects of North America. *Edited by R. W. Merritt and K. W. Cummins. Kendall/Hunt Publishing Co., Dubuque, Iowa. 1978. xiii + 441 p. $21.95.*

This volume of some 22 chapters (17 taxonomic and ecological, 5 introductory or general), individually or jointly authored by some of the best known workers in freshwater ecology and taxonomy, is well organized and edited. In general, the figures are clearly labeled and useful. In fact, from the preprint advertising and at first glance, it appears to be the answer to the aquatic biologists' prayers.

However, on closer examination it is obvious that although it will be of great use to some, the book has some serious limitations. The first, and perhaps the most important of these, is that most of the taxonomic keys (chapters 6–22, and therefore most of the book!) except those to the Tipulidae, Culicidae, and Simuliidae (chapters 19, 20 and 21) are only to the family or tribe taxonomic level. Thus, they are not only available in many other books, but also they are of very limited use to aquatic biologists in general. Perhaps the saving grace of these chapters is the excellent tables summarizing the known ecology, distribution, etc., and references to the sources of these data at the generic level. It is interesting to note that, in many cases, these tables were not prepared by the author(s) of the chapter.

The introductory chapters (1–5) are informative and well written, particularly the chapter on sampling methods, but suffer in some instances in being too detailed. It seems unnecessary, for instance, to explain that the dorsal side of an insect is the top, or the ventral, the bottom. Also, the use of summary tables throughout, without discussion of the relative merits of the works listed in these tables, greatly detracts from the value of the book as a teaching or research aid. This is particularly true in the sampling methods section, where a discussion of the research works referred to should lead the authors to recommend a particular sampling apparatus, for a given habitat, rather than just listing all of the possibilities.

In general, it appears that the editors or authors, by aiming at such a wide audience (fishermen, students, professional biologists, etc.), have diluted their offering to the point where the book has very limited appeal. However, in spite of the above, the book is well worth the very reasonable price since it is an outstanding reference book to most areas of freshwater ecological research.

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(R240)

Journal of Fish Diseases, Vol. 1, No. 1. *Edited by R. J. Roberts. Blackwell Scientific Publications, Oxford, England. 1978. 136 p. $50.00 subscription (USA and Canada).*

This new international journal is designed to fulfill a need for a comprehensive journal of fish and shellfish diseases. In the Foreword Dr Snieszko rightfully supports the need for such a publication in English, and he mentions the hope that this periodical will help to initiate the formation of an international organization of fish pathologists, with the *Journal of Fish Diseases* eventually becoming its official organ. With such a significant dedication, it would be helpful if abstracts of articles were provided in two international languages. Nevertheless, the *Journal* editorial board is outstanding, and the editor and associate editor have admirably demonstrated their collective judgment and skill in the selection of an excellent series of high quality articles for this first issue.

Among the significant articles that appear is a paper by H. W. Ferguson and E. A. Needham describing a new form of salmonid kidney disease, proliferative kidney disease (PKD). The argument is presented that the condition is attributable to infection by a parasite, possibly an amoeba, in combination with poor quality water. Mortalities can be extensive, and more recent reports indicate that the disease is now a recognizable problem in a number of European countries. In another article, N. Peters and his coworkers in collaboration with H. F. Stich and A. B. Acton of the University of British Columbia demonstrate that the skin tumors of the Atlantic flatfishes are histologically different from the complex virus-associated papillomas found in the Pacific flatfishes. Later in this same issue I. B. Duncan describes a possible tumor virus of salmonids, when he presents evidence for the existence of an oncovirus in swimbladder fibrosarcomas of Atlantic salmon (*Salmo salar*).

An intriguing article by J. S. Buchanan describes a rickettsia-like organism found in association with a phage in the digestive gland of the marine bivalve mollusc *Tellina tenuis*. This is believed to be the first description of a virus from a rickettsia. The icosahedral virus particles were apparently significantly different in size and buoyant density

from the virus isolates from *T. tenuis* which were previously found to be morphologically and biochemically similar to infectious pancreatic necrosis virus (Underwood et al., J. Gen. Virol. 36: 93–109, 1977).

A study by E. C. Egidius and K. Andersen on Norwegian isolates of *Vibrio anguillarum* indicates that strains isolated from diseased rainbow trout (*S. gairdneri*) were pathogenic to salmonids and probably to a very low extent to saithe (*Pollachius virens*). In contrast, strains isolated from saithe were pathogenic to saithe, but not to salmonids. The authors indicate that their observations support the validity of grouping *V. anguillarum* isolates on a host-of-origin basis.

Additional articles of interest cover such diversified aspects of fish pathobiology as tissue reactions of the three-spined stickleback (*Gasterosteus aculeatus*) to infection by *Glugea anomala, Saprolegnia* infections in salmonids, heat stability of antibody of coho salmon (*Oncorhynchus kisutch*), the pathogenesis of streptococcal infections in cultured yellowtails (*Seriola quinqueradiata* and *S. purpurascens*), and the development of skin lesions associated with extended treatment of channel catfish (*Ictalurus punctatus*) with nifurpirinol (Furanace).

Although there are a number of established publications (including the *Journal of the Fisheries Research Board of Canada*) which will continue to be in direct competition for high quality articles on fish diseases, the *Journal of Fish Diseases* appears certain of a successful future. Furthermore, there are currently no page charges for contributors to this quarterly journal. Delivery to North American subscribers is by airfreight, thus assuring that copies are generally received within 2 weeks of publication. Most certainly all fisheries-oriented libraries will wish to subscribe to this excellent new periodical.

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(R224)

Books Received/Livres reçus

July 1978

Guide to the Study of Animal Populations. *James T. Tanner. University of Tennessee Press, Knoxville, 1978. xiv + 186 p. $8.95.* (A small, but very densely packed book on various aspects of population studies, especially the attributes of populations underlying population dynamics, species interactions, etc.)

September 1978

***Development of Fishes of the Mid-Atlantic Bight — An atlas of Egg, Larval and Juvenile Stages.**

Vol. I. Acipenseridae through Ictaluridae. P. W. Jones, F. D. Martin and J. D. Hardy, Jr. 366 p.

Vol. II. Anguillidae through Syngnathidae. J. D. Hardy, Jr. 458 p.

Vol. III. Aphredoderidae through Rachycentridae. J. D. Hardy, Jr. 394 p.

Vol. IV. Carangidae through Ephippidae. G. D. Johnson. 314 p.

Vol. V. Chaetodontidae through Ophidiidae. R. A. Fritsche. 340 p.

Vol. VI. Stromateidae through Ogcocephalidae. F. D. Martin and G. E. Drewry. 416 p. U.S. Fish and Wildlife Service. 1978. $62.25/set.

(Available from Superintendant of Documents, U.S. Government Printing Office, Washington, D.C. 20402)

Salmon and Trout Farming in Norway. *David J. Edwards. Fishing News Books Ltd., Farnham, Surrey, England. 1978. xii + 195 p. £9.00.* (A small book covering many aspects of Norwegian aquaculture, including food, diseases, economics, etc.)

Food Webs and Niche Space. *Joel E. Cohen. Monographs in Population Biology 11. Princeton University Press, Princeton, N.J. 1978. xv + 189 p. Cloth — $14.00, paper — $6.95.* (This book presents a technique for determining the minimum dimension of a niche space, based on a relation between the combinatorial structure of food webs and the mathematical theory of interval graphs.)

Third Annotated Bibliography on Biological Effects of Metals in Aquatic Environments *(No. 1293-2246). Ronald Eisler, Daniel J. O'Neill, Jr. and Glen W. Thompson. U.S. Environmental Protection Agency, Narragansett, RI 02882. 1978. vi + 487 p.* (Available from National Technical Information Service, 5285 Port Royal Road, Springfield, V.A. 22161. $15.00.)

Coral Reefs: Research Methods. *Edited by D. R. Stoddart and R. E. Johannes. UNESCO, 7 Place de Fontenoy, Paris. 1978. xv + 581 p.* (This monograph is an update of the Handbook for Atoll Research by Fosberg and Sachet, 1953 except that it is now limited to the aquatic environment.)

October 1978

***An Introduction to the Aquatic Insects of North America.** *Edited by Richard W. Merritt and Kenneth W. Cummins. Kendall/Hunt Publishing Co., Dubuque, Iowa. 1978. xiii + 441 p. $21.95. (See review in this issue.)*

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[1]Entries in this list *not* marked by an asterisk are available for review by interested readers for a 3-month period from the time of publication of the list. For information, please write to

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CORRECTIONS TO RECENT PUBLICATIONS
CORRECTIONS SUR RÉCENTES PUBLICATIONS

VOLUME 34, No. 8 — paper by W. T. Momot and H. Gowing: *Response of the crayfish Orconectes virilis to exploitation.*

Page 1218: Table 6, data for North Twin Lake 1967–68 should be

♂ Age-group		♀ Age-group	
0	17.7	0	17.7
1	40.7	1	27.2
2	3.5	2	4.8
3	2.6	3	0.2
	64.5		49.9

VOLUME 35, No. 5 — paper by J. J. Stegeman: *Influence of environmental contamination on cytochrome P-450 mixed-function oxygenases in fish: implications for recovery in the Wild Harbor Marsh.*

Page 668: Abstract, line 4, "aminopyrene" should be "aminopyrine"; Résumé, lines 2 and 5, "aminopyrène" should be "aminopyrine".

VOLUME 35, No. 7 — paper by M. Yurkowski, J. K. Bailey, R. E. Evans, J.-A. L. Tabachek, G. B. Ayles, and J. G. Eales: *Acceptability of rapeseed proteins in diets of rainbow trout (Salmo gairdneri).*

Page 958: Figure 5, y axis, "plasma T_4 (ng/g)" should be "plasma T_4 (ng/dL)".

VOLUME 35, No. 7 — paper by G. A. McFarlane and W. G. Franzin: *Elevated heavy metals: a stress on a population of white suckers, Catostomus commersoni, in Hamell Lake, Saskatchewan.*

Page 966: Right column, par. 3, line 5, "$(0.025 < P > 0.010)$" should be "$(0.025 > P > 0.010)$".

VOLUME 35, No. 7 — note by R. W. M. Hirtle and K. H. Mann: *Distance chemoreception and vision in the selection of prey by American lobster (Homarus americanus).*

Page 1006: Received and accepted dates should be as follows:

Received February 2, 1978	Reçu le 2 février 1978
Accepted April 3, 1978	Accepté le 3 avril 1978

VOLUME 35, No. 7 — Outside back cover, Book Reviews/Comptes rendus, replace **"J. W. Gibbons and R. R. Sharitz"** with **"J. W. McMahon"**.

COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN

Uptake from seawater and clearance of *p, p'*-DDT by marine planktonic crustacea
G. C. H. Harding and W. P. Vass

Lightweight corer designed for sampling very soft sediments
J. D. H. Williams and A. E. Pashley

Concentrations of Cd, Cu, Mn, Pb, and Zn in fishes in a highly organic softwater pond
J. G. Wiener and J. P. Giesy Jr.

A reason for pleomerism **J. L. Spouge and P. A. Larkin**

Notes

Role of phosphorus in Great Lakes eutrophication: Is there a controversy? **C. L. Schelske**

Iodine balance in rainbow trout (*Salmo gairdneri*) and effects of testosterone propionate
D. W. C. Hunt and J. G. Eales

Comparative susceptibility of Atlantic (*Salmo salar*) and coho (*Oncorhynchus kisutch*) salmon to three strains of *Vibrio anguillarum* from the Maine–New Hampshire coast
E. S. Sawyer, R. G. Strout, and B. A. Coutermarsh

Perspectives

Freshwater Benthic Invertebrate Life Histories: Current Research and Future Needs

Introduction **D. M. Rosenberg**

Sampling variability and life history features: basic considerations in the design of aquatic insect studies **V. H. Resh**

Sampling strategies and life history of non-insectan freshwater invertebrates
D. F. Malley and J. B. Reynolds

Contribution of life history information to taxonomy of aquatic insects **D. R. Oliver**

Use of macroinvertebrate life history information in toxicity tests
A. L. Buikema Jr. and E. F. Benfield

Environmental disturbance and life histories: principles and examples **D. M. Lehmkuhl**

Influences of diet on the life histories of aquatic insects
N. H. Anderson and K. W. Cummins

Benthic life histories: summary and future needs **T. F. Waters**

RECENT PUBLICATIONS / RÉCENTES PUBLICATIONS

The number following each title in each series refers to the establishments listed at the end of the section. Inquiries concerning any particular item should be directed to the issuing establishment.

Les nombres qui suivent les titres de chacune des séries désignent les établissements suivants à la fin de la section. Pour toute demande de renseignements concernant un rapport particulier, il faut s'adresser au service responsable.

Recent Studies

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles on investigations published in other periodicals, which henceforth may be obtained in reprint form only from the author or originating establishment.

Récentes études

Jusqu'en 1970, ces articles étaient réimprimés, puis reliés dans un volume annuel d'études, comprenant deux parties. Les études de 1969, partie 2, incluant le n°1355, ont été les dernières à être reliées. Nous n'en faisons désormais plus la reliure ni la distribution. Nous continuons cependant de numéroter les articles et d'en établir des listes d'études sur des recherches mentionnées dans d'autres périodiques. On peut donc se les procurer, sous leur forme réimprimée seulement, de l'auteur ou du service dépositaire.

2538. LANDYMORE, A. F., AND N. J. ANTIA. 1978. White-light promoted degradation of leucopterin and related pteridines dissolved in seawater, with evidence for involvement of complexation from major divalent cations of seawater. Mar. Chem. 6: 309–325. (3)

2539. MUIR, D. C. G., AND BRUCE E. BAKER. 1978. The disappearance and movement of three tiazine herbicides and several of their degradation products in soil under field conditions. Weed. Res. 18: 111–120. (4)

2540. BOHN, A., AND B. W. FALLIS. 1978. Metal concentrations (As, Cd, Cu, Pb and Zn) in shorthorn sculpins, *Myoxocephalus Scorpius* (Linnaeus), and artic char, *Salvelinus alpinus* (Linnaeus), from the vicinity of Strathcona Sound, Northwest Territories. Water Res. 12: 659–663. (4)

2541. HARRISON, W. G. 1978. Experimental measurements of nitrogen remineralization in coastal waters. Limnol. Oceanogr. 24: 684–694. (7)

2542. HEALEY, M. C. 1978. The dynamics of exploited lake trout populations and implications for management. J. Wildl. Manage. 42: 307–328. (1)

2543. ROSENBERG, D. M., AND A. P. WIENS. 1978. Effects of sediment addition on macrobenthic invertebrates in a Northern Canadian River. Water Res. 12: 753–763. (4)

2544. SIPOS, J. C., AND R. G. ACKMAN. 1978. Automated and rapid quantitative analysis of lipids with chromarods. J. Chromatogr. Sci. 16: 443–447. (8)

2545. KE, P. J., AND A. D. WOYEWODA. 1978. A titrimetric method for determination of free fatty acids in tissues and lipids with ternary solvents and m-cresol purple indicator. Anal. Chim. Acta. 99: 387–391. (8)

2546. ACKMAN, R. G., AND J. MCLACHLAN. 1978. Fatty acids in some Nova Scotian marine seaweeds: a survey for octadecapentaenoic and other biochemically novel fatty acids. Proc. N.S. Inst. Sci. 28: 47–64. (8)

2547. HARA, T. J., AND B. E. THOMPSON. 1977. The reaction of whitefish, *Coregonus clupeaformis*, to the anionic detergent sodium lauryl sulphate and its effects on their olfactory responses. Water Res. 12: 893–897. (4)

2548. LANDYMORE, A. F., N. J. ANTIA, AND G. H. N. TOWERS. 1978. The catabolism of L-phenylalanine, L-tyrosine, and some related aromatic compounds by two marine species of phytoplankton. Phycologia 17: 319–328. (3)

2549. DEMARCH, B. G. E. 1978. The effects of constant and variable temperatures on the size, growth, and reproduction of the freshwater amphipod *Hyalella azteca* (Saussure). Can. J. Zool. 56: 1801–1806. (4)

2550. SEBEDIO, J.-L., AND R. G. ACKMAN. 1978. Oxidative ozonolysis of a polyunsaturated fatty acid in BF_3-MeOH medium. Can. J. Chem. 56: 2480–2485. (8)

2551. BERNARD, F. R. 1978. New bivalve molluscs, subclass Pteriomorphia, from the northeastern Pacific. Jpn. J. Malacol. 37: 61–75. (1)

2552. LANDYMORE, A. F., AND N. J. ANTIA. 1978. White-light promoted degradation of leucopterin and related pteridines dissolved in seawater, with evidence for involvement of complexation from major divalent cations of seawater. Mar. Chem. 6: 309–325. (1)

2553. SUNDBY, B., AND D. H. LORING. 1978. Geochemistry of suspended particulate matter in the Saguen Fjord. Can. J. Earth Sci. 15: 1002–1011. (7)

2554. LORING, D. H. 1977. Geochemistry of zinc, copper, and lead in the sediments of the estuary and Gulf of St. Lawrence. Can. J. Earth Sci. 15: 757–772. (7)

2555. SILVER, W., AND T. PLATT. 1978. Energy flux in the pelagic ecosystem: a time-dependent equation. Limnol. Oceanogr. 23: 813–816. (7)

2556. BOWERING, W. R. 1978. Age and growth of the Greenland halibut, *Reinhardtius hippoglossoides* (Walbaum), in ICNAF subareas 2–4. Int. Comm. Northwest Atl. Fish. Res. Bull. (13): 5–10. (9)

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2558. SHAW, D. H., AND H. J. HODDER. 1978. Lipopolysaccharides of the motil aeromonads: core oligosaccharide analysis as an aid to taxonomic classification. Can. J. Microbiol. 24: 864–868. (9)

2559. DAWE, L. L., AND W. R. PENROSE. 1977. "Bactericidal" property of seawater: death or debilitation? Appl. Environ. Microbiol. 35: 829–833. (9)

Translation Series

The following translations have been prepared by or for the Fisheries and Marine Service, for use in its investigations. Copies are available from Translation Services, Canada Institute for Scientific and Technical Information, National Research Council of Canada, Ottawa, Ontario K1A 0S2, at a cost of 22¢ per page and a minimum charge of $2.20. Information on translations can be obtained from the Scientific Information and Publications Branch.

Traductions

Les traductions dont il est question ci-après ont été préparées par ou pour le Service des pêches et des sciences de la mer, à des fins de recherche. On peut en obtenir des exemplaires, à 22¢ la page et à un prix minimum de $2.20 aux Services de traduction, Institut canadien de l'information scientifique et technologique, Conseil national de recherches du Canada, Ottawa (Ontario), K1A 0S2. La Direction de l'information et des publications scientifiques peut fournir des renseignements sur ces traductions.

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ANNOUNCEMENTS / AVIS

International Conference on Fish Science and Technology

This conference will be held at the Natural Philosophy Department, University of Aberdeen, Aberdeen, Scotland, from July 23 to 27, 1979. Session 1 will consider the past, present, and future of fish technology. Session 2 will consider the past, present, and future of fish science. Both sessions will be plenary lectures given by invited speakers. Sessions 3 and 4, run concurrently, will be devoted to contributed papers. All papers will be presented in English. Participants are invited to submit abstracts of original work before February 28, 1979. If accepted, authors will be asked during March to prepare a finished paper by the time of the conference. Send abstracts for contributed papers and requests for registration application forms (to be returned by May 31, 1979) to *Dr J. J. Connell, Torry Research Station, 135 Abbey Road, Aberdeen AB9 8DG, Scotland.*

Conférence internationale sur la science et de la technologie du poisson

Cette conférence aura lieu au Département de philosophie naturelle, Université d'Aberdeen, Aberdeen, Écosse, du 23 au 27 juillet 1979. La séance 1 considérera le passé, présent et futur de la technologie du poisson. La séance 2 considérera le passé, présent et futur de la science du poisson. Ces deux séances consisteront en des conférences plénières données par des orateurs invités. Les séances 3 et 4, qui auront lieu concurremment, seront consacrées à la présentation de contributions. Tous les travaux seront présentés en anglais. On demande aux participants de soumettre un résumé de leurs travaux originaux avant le 28 février 1979. Pour les travaux qui seront acceptés, on demandera aux auteurs au cours du mois de mars de préparer un article dans sa forme finale à temps pour la conférence. On est prié de transmettre les résumés de contributions et les demandes de formules d'enregistrement (devant être retournées avant le 21 mai 1979) au *Dr J. J. Connell, Torry Research Station, 135 Abbey Road, Aberdeen AB9 8DG, Écosse.*

Geometronics '79 Seminar Series

A series of four seminars organized and directed by Humber College of Applied Arts and Technology in cooperation with the Canadian Institute of Surveyors, Canadian Hydrographic Service, Canadian Hydrographers Association, and the Ontario Land Surveyors will have a predominantly hydrographic theme. The seminars are

I. Hydrography in Toronto May 7–11, 1979;
II. Sonar Workshop in Toronto May 7–10, 1979;
III. Law of the Sea in Ottawa May 14, 1979; and
IV. Control Surveys (Manor Program) in Toronto May 28–June 1, 1979.

For further information, please contact *Conference & Seminar Services, Humber College of Applied Arts & Technology, P.O. Box 1900, Rexdale, Ontario M9W 5L7, (416) 675-3111.*

Série de colloques '79 sur la géométronique

Lors d'une série de quatre colloques organisés et dirigés par le Humber College of Applied Arts and Technology, en coopération avec l'Institut canadien des arpenteurs-géomètres, le Service hydrographique canadien, l'Association canadienne des hydrographes et les arpenteurs de l'Ontario, on discutera surtout d'hydrographie. Les colloques sont les suivants

I. Hydrographie — Toronto, 7–11 mai 1979;
II. Atelier de travail sur le sonar — Toronto, 7–10 mai 1979;
III. Droit de la mer — Ottawa, 14 mai 1979; et
IV. Relevés de contrôle (Programme « Manor ») — Toronto, 28 mai–1er juin 1979.

Pour plus de renseignements, on est prié de s'adresser à *Conference & Seminar Services, Humber College of Applied Arts & Technology, P.O. Box 1900, Rexdale, Ontario M9W 5L7, (416) 675-3111.*

Third International Congress on the History of Oceanography

Call for Papers

The Third International Congress on the History of Oceanography will be held September 22–26, 1980 at Woods Hole, Massachusetts, USA. Please submit offers of contributions as soon as possible. Abstracts of the proposed contributions, not exceeding 500 words and in English, are due not later than March 31, 1979. An original copy of each accepted paper, in double-spaced typescript with ample margins, will be required by August 31, 1979. Publication of the papers will be in a special issue of the *Journal of Marine Research,*

Please send offers of contributions to *Dr Daniel Merriman, Chairman of the Organizing Committee, 298 Sperry Road, Bethany, Connecticut 06525, USA.*

Troisième congrès international sur l'histoire de l'océanographie

Appel d'articles

Le Troisième congrès international sur l'histoire de l'océanographie se tiendra à Woods Hole, Massachusetts, É.-U., du 22 au 26 septembre 1980. On est prié de soumettre le plus tôt possible les offres de contributions. Les résumés des contributions proposées, ne dépassant pas 500 mots et rédigés en anglais, doivent nous parvenir avant le 31 mars 1979. On devra produire avant le 31 aout 1979 un original de chaque article accepté, tapé à la machine à deux interlignes avec amples marges. Les articles seront publiés dans un numéro spécial du *Journal of Marine Research.*

On est prié d'adresser les offres de contributions à *Dr Daniel Merriman, Chairman of the Organizing Committee, 298 Sperry Road, Bethany, Connecticut 06525, USA.*

S.I.L. Workshop on Hypertrophic Ecosystems

Preliminary Notice and Call for Papers

The Institute of Limnology, University of Lund, will host in Växjö, Sweden, a limnological workshop on hypertrophic ecosystems. The workshop, under the auspices of the Societas Internationalis Limnologiae will be held from September 10–14, 1979. The format will include round-the-table presentations by invited speakers, contributed papers, and floor discussions in four joint sessions. The number of presentations will not exceed 50. One day will be reserved for field excursions to former hypertrophic lakes that are now restored.

The objectives of the workshop are to seek better understanding of highly eutrophic, disturbed, and unstable ecosystems. These include lakes, reservoirs, and ponds that have developed noxious algal and bacterial blooms, shown extreme fluctuations in their water quality on a daily and seasonal scale, produced gases, off-flavor, and toxic substances, and experienced periodic anoxia and massive fish kills, i.e. systems requiring nonconventional corrective measures and new concepts for their solution beyond those generally accepted for eutrophic systems.

For more information write to *J. Barica, Program Chairman, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba R3T 2N6.*

Atelier de travail de l'Institut de limnologie de Suède sur les écosystèmes hypertrophes

Avis préliminaire et appel d'articles

L'Institut de limnologie de l'Université de Lund sera l'hôte d'un atelier de travail limnologique sur les écosystèmes hypertrophes à Växjö, Suède. L'atelier de travail, sous les auspices de la Societas Internationalis Limnologiae, aura lieu du 10 au 14 septembre 1979. Il y aura présentations, en table ronde, par des spécialistes invités, des contributions et des discussions générales durant les quatre séances conjointes. Les présentations ne dépasseront pas 50. On a réservé une journée à des excursions à des lacs qui étaient jadis hypertrophes et qui ont été restaurés depuis.

Les objectifs de l'atelier de travail sont de mieux comprendre les écosystèmes fortement eutrophes, dérangés et instables. Ce sont les lacs, les réservoirs et les étangs qui ont produit des fleurs d'eau d'algues et de bactéries nuisibles, ont montré des fluctuations extrêmes, journalières ou saisonnières, de la qualité de l'eau, ont produit des gaz et des substances toxiques et de saveur désagréable et qui, enfin, ont subi une anoxie périodique et des mortalités massives de poissons, i.e. des systèmes qui, pour être corrigés, ont besoin de mesures non conventionnelles et de nouveaux concepts dépassant ceux qui sont généralement acceptés pour des systèmes eutrophes.

Pour plus amples renseignements, veuillez vous adresser à *J. Barica, président du programme, Institut des eaux douces, 501 University Crescent, Winnipeg, Manitoba R3T 2N6.*

REVUE CANADIENNE DE BIOLOGIE

Directeur / *Editor* : Dr M.-Ather Ali

Ce périodique bilingue fondé il y aura bientôt quarante ans, par quelques éminents biologistes canadiens-français en vue de diffuser les résultats de leurs travaux de recherche, publie des articles originaux dans les domaines de la biologie et médecine expérimentales, notamment la morphologie fonctionnelle, la physiologie sensorielle et environnementale.

This bilingual periodical, founded almost forty years ago by a group of eminent French-Canadian Biologists as an outlet for the results of their investigations, aims to publish original papers in Experimental Biology and Medecine including Functional Morphology, Environmental and sensory Physiology.

Trimestrielle, elle paraît en mars, juin, septembre et décembre. Les abonnements (1er janvier - 31 décembre) commencent avec le premier numéro de l'année. / *Issued quarterly : March, June, September and December. Every yearly subscription (January 1st December 31st) starts with the first issue.*

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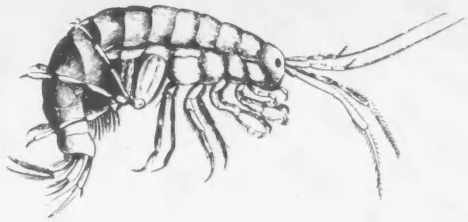

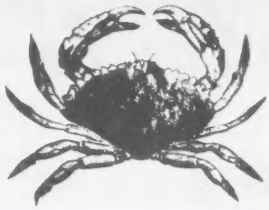

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AQUACULTURE is an international journal devoted to research on the exploration, improvement and management of all aquatic food resources, both floristic and faunistic, from freshwater, brackish and marine environments, related directly or indirectly to human consumption. Relevant topics include:

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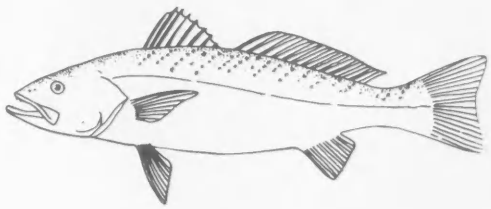

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